Model Annual Report

NIEHS Core Center Program
2001

Introduction

The purpose of this document is to provide assistance to NIEHS Core Centers as they write their electronic annual report. Selected summaries have been compiled from several Core Center CY2000 annual reports.

The document is organized as follows:

I. Center Summary (2 examples provided)
II. Research Highlights (2 examples provided)
III. Administrative Core (1 example provided)
IV. Research Cores (2 examples provided)
V. Facility Cores (3 examples provided)
VI. COEP (2 examples provided)
VII. Pilot Project (2 examples provided)

In some instances more than one Center has been used as an example for a particular section. The reasons for having more than one example are to give Centers a sense of the variety of ways a section can be written and in some instances allow for the natural differences in Facility and Research Cores. In other words, there is not necessarily a "one size fits all" way of writing a section.

Questions regarding this document should be directed to Mr. Liam O'Fallon.

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Center Summary

Example #1.

Introduction: The goal of the University of Rochester NIEHS Environmental Health Sciences Center is to define the impact of and understand the mechanisms whereby exposures to environmental and occupational agents contribute to human disease and dysfunction. Achievement of this goal not only provides a sound experimental basis for assessment of the risk(s) environmental and occupational agents pose to human populations, but they may also be critical to the formulation of preventive strategies. These goals are achieved through interdisciplinary collaborations among Center faculty in four Research Cores and enhanced through Center resources, including the Facilities Cores and the Enrichment Program.

Research Cores: The Neurotoxicology Core focuses on the extent to which toxicants act as risk factors for diseases and dysfunctions of the nervous system, particularly on the interaction between environmental factors and genetic predisposition. The Pulmonary Toxicology Core addresses mechanisms of lung injury and repair and how these are modulated by underlying disease processes. The Protein Modulators of Toxicity Core focuses on biologically active proteins that critically regulate molecules in normal cells as being molecular targets of toxicity. The Osteotoxicology Core addresses the impact of environmental and occupational agents on mineralized tissue, in particular the roles of lead in caries in children, and the extent to which lead exposure serves as a risk factor for osteoporosis and related skeletal diseases associated with advancing age.

Facilities Core: The Facilities Cores of the University of Rochester National Institute of Environmental Health Sciences Center are designed to enhance the research capabilities and directions of the Research Cores. The Shared Instrument Facility Core includes equipment purchased and maintained by the Center for faculty use. It includes assistance with use of equipment and development of assays. The Biostatistics Facility Core is critical for both human cohort studies as well as experimental efforts. The structure of the Core allows faculty to work with various members of the Department of Biostatistics. The Pathology/Morphology/Imaging Facility Core offers assistance with assessment of pathological and morphological changes. Imaging facilities have been upgraded to include static confocal and fluorescent imaging, as well as a PixCell laser capture micro dissection instrument. The Transgenic Services Facility Core offers Center faculty assistance in the generation of genetically-engineered mice, and capabilities for cryopreservation and rederivation. Our University Facility Core offers Center faculty financial resources to utilize Cores developed by the University of Rochester Medical Center, including flow cytometry, microarray technology, protein sequencing.

Community Outreach and Education: The Community Outreach and Education Program (COEP) of the University of Rochester National Institute of Environmental Health Sciences Center focuses primarily on educational programs aimed at students of various levels as well as a growing number of teacher training programs. Educational activities are primarily hands-on programs with Center faculty involvement as well as the development of new teaching materials, including science kits for classroom use. A Community Advisory Board serves as a conduit for information exchange of Center-based research into the community and community issues to the Center.

Pilot Projects: The pilot project program is a component of the Center's Enrichment program and serves as an extremely successful mechanism whereby the Center has expanded its research directions and brought new faculty into the Center. Pilot projects are available to all faculty at the University of Rochester but priority is given to projects that are congruent with the theme of the Center, i.e. environmental and occupational agents as modulators of human disease and dysfunction. Eight projects were awarded over this past year. Data obtained from four of these projects have already led to successful extramural funding, and grants based on data generated by another are now under review.

Example #2

Introduction

The University of Iowa Environmental Health Sciences Research Center was established in 1990 with funding from the National Institute of Environmental Health Sciences. It serves as the Environmental Health Research Center in the rural Midwest, the heart of America's most productive agricultural region. Rural Americans are more often at increased risk from a number of agricultural and rural environmental hazards and exposures, have less access to health care providers, and generally have more adverse health outcomes than Americans living in urban areas. Environmental health problems affecting rural populations have received far less attention than environmental health concerns facing urban residents. Farmers, farm family members, children, the elderly, and the poor are at particular risk for rural and environmental diseases. Asthma prevalence among rural children is nearly three times the national average, certain cancers associated with pesticide exposures are common, and rural residents are exposed to pesticides, fertilizers and organic dust leading to increased prevalence of lung ailments. As the second most rural state in the nation, Iowa provides an excellent location in which to study relationships between rural environmental exposures and health outcomes.

The goals of the Environmental Health Sciences Research Center are to operate an interdisciplinary environmental health research center with a focus on agricultural and rural environmental exposures and health effects, and to promote research interactions between environmental health researchers at The University of Iowa, enhancing ongoing environmental health research and facilitating initiation of new collaborative and interdisciplinary environmental health research.

Environmental Health Sciences Research Center investigators demonstrate national and international leadership in the areas of environmental cancer, pulmonary biology, occupational health, and environmental assessment and control. In its capacity as a technical resource for legislative bodies, grassroots community organizations, and populations with special needs, the Environmental Health Sciences Research Center serves the people of the Midwest and other rural populations. The following Research Cores and accompanying Facility Cores are designed to address the needs of the region using expertise within The University of Iowa.

Research Cores

Environmental Epidemiology Research Core: Conducts research on cancer and adverse reproductive outcome etiology, prevention and control; performs epidemiologic, biostatistical and statistical genetics methods research.
Environmental Assessment and Control Research Core: Performs original research in the area of rural environmental exposure assessments with a secondary goal of providing intervention and engineering remediation of rural and agricultural problems.
Occupational Health Research Core: Conducts research on agricultural health and injury prevention related to rural and occupational exposures.
Pulmonary Biology Research Core: Develops new science related to environmental lung diseases, especially as it relates to agricultural exposures.

Facility Cores

Clinical Exposure Facility Core: Promotes clinical research studies on relevant agricultural and rural exposures and their health effects through its high quality, dedicated facility.
Exposure Assessment Facility Core: Enhances research through provision of state-of-the-art exposure assessment services, including chemical, microbiological and physical agents in all media.
Health Registry Facility Core: Provides data and service through the dedicated, experienced personnel of the Iowa Cancer Registry, Iowa Birth Defects Registry, Center for Health Effects of Environmental Contamination (CHEEC), and Rural Injury Surveillance System (RISS).
Inhalation Toxicology Facility Core: Provides facilities and expertise for exploring original research areas in pulmonary biology, inhalation toxicology, or aerosol science.

Community Outreach and Education Core

In an ongoing effort to address the environmental concerns of Iowans and others living in the Midwest, the Environmental Health Sciences Research Center's Community Outreach and Education Core draws together representatives from colleges and universities, K-12 schools, agribusiness, state legislative bodies, and

grassroots rural and environmental organizations. The Core serves to channel the diverse environmental health capabilities of the Environmental Health Sciences Research Center and its affiliated research units at The University of Iowa toward meeting the needs of the broader community in terms of educational support, health promotion and outreach, as well as serving the upper Midwest as a technical resource to assist in environmental health policy making.

Pilot Project Grant Program

The Pilot Grant Program continues to play an important role in providing seed funding primarily to junior investigators who go on to compete successfully for full funding with submissions to NIH, NSF, CDC, HUD, and a wide variety of foundations. Any faculty member with a research interest in environmental health consistent with the goal of the Center is eligible to apply for pilot project grant support. Priority is given to funding young investigators. There currently is the regular Research Pilot Grant Program and the International Pilot Grant Program. Over the past four years an influx of $335,000 to support pilot grants has generated 25 extramural grants with a total dollar amount of over $6.5 million. In addition, over 42 peer-reviewed publications emanated from the 26 pilot grants. In 2000, 4 research pilot grants and 3 international pilot grants were funded.

Research Highlights

Example #1

Title: Combined Paraquat and Maneb Exposure: Implications for Parkinson's Disease.

Significance: Parkinson's disease was first described in 1817 by James Parkinson, a British physician who published a paper on what he referred to as "the shaking palsy". Ever since Dr. Parkinson identified this disease, researchers have been trying to identify and find a treatment for it. The disease is a motor system disorder and is both chronic and progressive. Previous studies have explored the hypothesis that environmental agents may have a relationship to the disease and have led to findings that pesticides might provide a clue. Recent studies by Dr. Deborah Cory-Slechta and others within the Neurotoxicology Core have found a combination of chemicals that can be linked to Parkinson's disease. The research studied the effects of paraquat, a weed killer sprayed aerially on cotton and food crops, and maneb, a pesticide used on farms and home gardens, in mice. They found that doses of these compounds, which were without effect when given alone, had potentiated effects when given in combination. This combination also targeted the nigrostriatal dopamine system, the same neurochemical system that undergoes degeneration in Parkonson's disease. Subsequent studies have shown that the combination of paraquat and maneb has a greater impact in aging animals and that its adverse effects on the nigrostriatal dopamine system are not reversible. Notably, risk assessment guidelines for human exposure to these and other compounds are typically based on levels producing no effects derived from exposure to single agents. The findings show, however, that such compounds, while having no or marginal effects when administered individually, can produce synergistic effects when given in combination. This suggests that the current derivation of risk assessment guidelines needs to be evaluated. The studies were in collaboration with Drs. Eric Richfield and Ray Baggs of the Center and Dr. A.W. Tank from the Department of Pharmacology. These investigations were also assisted by Pilot Project funds and the Center's Pathology/Morphology/Imaging Facility Core.

Publications:

Thiruchelvam, M., Richfield, E.K., Baggs, R.B., Tank, A.W., and Cory-Slechta, D.A. 2000. The nigrostriatal dopaminergic system as a preferential target of repeated exposures to combined paraquat and maneb: Implications for Parkinson's disease. J. Neuroscience 15: 9207-9214.

Example #2

Development of a New Mouse Model for Prostate Cancer

Prostate cancer is the most commonly diagnosed cancer in men in the United States. Progress in prostate disease research has been impaired by the lack of adequate animal models that reproduce the human disease. Recent research by CRED scientists has resulted in the development and characterization of transgenic mice expressing human insulin-like growth factor 1 (IGF-1) in basal epithelial cells of prostate. Transgene expression led to activation of the IGF-1 receptor (IGF-1r) and prostate tumor development in older mice. The incidence of tumors (well differentiated adenocarcinomas) in mice $\geq$ 6 months of age was 50%. The development of prostate tumors in these transgenic mice appeared to follow a stepwise progression through abnormal cell growth that ultimately culminated in malignant tumors. These transgenic mice appear to represent a new animal model for human prostate cancer. The current results also provide a direct link between IGF-1r signaling and tumor development in prostate, confirming an important role for this growth factor in prostate cancer development. The further study of these mice should not only provide insight into the role of IGF-1r signaling in epithelial tumor formation but should also provide a greater understanding of its role in prostate cancer. In addition, these mice will be extremely useful for identifying potential new therapeutic and preventive strategies for this important human cancer.

Publications:

DiGiovanni, J., Bol, D. K., Wilker, E., Beltran, L., Carbajal, S., Moats, S., Ramirez, A., Jorcano, J., and Kiguchi, K. Constitutive expression of insulin-like growth factor-1 in epidermal basal cells of transgenic mice leads to spontaneous tumor promotion. Cancer Res. 60: 1561-70, 2000.

DiGiovanni, J., Kiguchi, K., Frijhoff, A., Wilker, E., Bol, D. K., Beltran, L., Moats, S., Ramirez, A., Jorcano, J., and Conti, C. Deregulated expression of insulin-like growth factor 1 in prostate epithelium leads to neoplasia in transgenic mice. Proc Natl Acad Sci U S A. 97: 3455-60, 2000.

Administrative Core

Example #1

The Director, Dr. Guengerich, is in charge of this unit. He is assisted by the Manager, A. R. Harrelson, and the Deputy Director, Dr. T. M. Harris, the latter particularly when he is out of town or is unable to attend Center Directors' meetings. The Director is appointed by the Dean of the School of Medicine. The Director also interacts with the Vice-Chancellor for Health Affairs and has input from the Steering Committee, the Research Core Leaders, the Facility Core Directors (all at Vanderbilt) and the External Advisory Group.

Description: The administrative cores include the Director's Office and Administrative Core. The Administrative Cores coordinate all aspects of the Center. The Center Investigators form the Center in Molecular Toxicology and are selected carefully. They derive benefits from the Center, in terms of support of their research activities, and have the responsibility of advancing environmental health research at Vanderbilt. Center Investigators are named by the Director and the Steering Committee, with the advice of the External Advisory Group. The number of Center Investigators is not expected to be large, as the policy is to involve a relatively small number of research-track faculty who will enhance the state of environmental health research by collaborating and encouraging others. A major benefit of limiting the number of Center Investigators is the maintenance of a cohesive and organized group. Criteria for selection as a Center Investigator include evidence of research independence, long-term commitment to research in areas related to environmental health, and the willingness to interact with others in a center environment. Only tenure track faculty are eligible. Ideally, Center Investigators will have already obtained independent peer-reviewed grant support and even the ability to renew this support, but new junior faculty will be considered if they have strong promise of success and can fill critical needs in the Center. Center Investigators are not selected with the view that they will rotate in and out of the Center but instead that they will remain active in environmental health science research and be a part of the Center throughout their careers at Vanderbilt. Center Investigators are expected to be able to influence research at Vanderbilt in their individual areas of interest. The Administrative Core also provides clerical and accounting service for the Center, the NIEHS training grant, and for the Center Investigators (Center-related business), with the help of Ms. Kathleen Trisler, Administrative Assistant, and Ms. Ellen Rochelle, Systems Administrator.

Internal Advisory Committee: The Steering Committee meets at least bimonthly, with many more frequent contacts by telephone and electronic mail, and is responsible for making major decisions, distributing pilot project funds, approving the operating budget, selecting the External Advisory Group, recommending the appointment and removal of Center Investigators, approving the competitive grant renewal applications, and selecting graduate students and postdoctoral trainees for the training grant (T32 ES07028). A. R. Harrelson (Center Manager) attends all meetings and keeps records. Members include:

Dr. F. P. Guengerich (Biochemistry), Director
Dr. T. M. Harris (Chemistry), Deputy Director
Dr. L. J. Marnett (Biochemistry, Chemistry)
Dr. D. G. Graham (Pathology)
Dr. M. R. Waterman (Biochemistry)

External Advisory Committee: The External Advisory Group consists of at least five individuals who visit Vanderbilt once each year (except during years when NIEHS site visits are held) and submits a formal report. In addition, members of the External Advisory Group are consulted for advice during the year, particularly with renewal applications and faculty recruitment. The members of the External Advisory Group are appointed to rotating terms by the Director and Steering Committee.

Dr. Marion W. Anders (Univ. of Rochester, Chairman)
Dr. Leona D. Samson (Harvard Univ.)
Dr. Michael L. Gross (Washington Univ.)
Dr. Kim Boekelheide (Brown Univ.)
Dr. Christopher Bradfield (Univ. of Wisconsin)

Research Cores

Example #1

Ecogenetics Research Core

A. Objectives

Ecogenetics Research Core objectives include: (a) identification of intraspecies genetic polymorphisms involving differences in response to environmental agents, (b) elucidation of the molecular genetic basis of these polymorphisms, (c) determination of the prevalence of different alleles in human populations and the mode of inheritance through family studies, (d) evaluation of the impact of a given polymorphism on human health, and (e) clinical pharmacogenetics and pharmacogenomics. The 1992 goals were principally directed toward single-gene (Mendelian) inheritance of alleles associated with environmental toxicity. Since 1994, members of this Core have focused on the importance of "multiplex phenotypes" (i.e. role of two or more genes involved in environmental toxicity or cancer), as well as closer integration with genomics and the Human Genome Project.

B. Core Director and Members

Daniel W. Nebert, M.D., Core Director, Professor, Department of Environmental Health
Marshall W. Anderson, Ph.D., Professor and Chairman, Department of Environmental Health
Iain L. Cartwright, Ph.D., Associate Professor, Department of Molecular Genetics, Biochemistry and Microbiology
Mary Beth Genter, Ph.D., Associate Professor, Department of Environmental Health
Anil G. Menon, Ph.D., Associate Professor, Department of Molecular Genetics, Biochemistry and Microbiology
S. Steven Potter, Ph.D., Professor, Department of Pediatrics, Developmental Biology
Jonathan S. Wiest, Ph.D., Assistant Professor, Department of Environmental Health

C. Key Words

Environmental diseases, Susceptibility genes, Heavy metal toxicity, Developmental gene expression, Genetic polymorphisms, Genetic epidemiology, Human populations, Multiplex phenotypes, Cardiovascular diseases, Pharmacogenetics

D. Progress

The members of this Core (including postdocs, graduate students, and technicians from each lab) meet on a formal basis a minimum of six times a year, discussing in turn (chalk talks highly encouraged) exciting breakthroughs that recently happened in their laboratories, a new technique or concept, or a proposed scientific collaboration that might promote enhanced interactions, between members within the Core—or other CEG members outside the Core. In addition, possible collaborations that might lead to a joint Pilot Project Program (PPP) application, joint R01 application, or Program Project Grant (PPG) application are constantly explored. There are also often discussions as to how best to use the CEG's F&S Cores. Such discussions and interactions also take place often by email (group e-mailing's), because this medium has been found to be an effective means of contacting everybody efficiently, getting input and thoughts expressed, and sharing creative ideas quickly.

Members of this Research Core are carrying out several collaborative human population studies of cancer or toxicity susceptibility genes/genomics. Studies are underway to isolate, identify and characterize a lung cancer tumor suppresor gene on the short arm of human chromosome (Chr) 9 (Anderson, Wiest). An ongoing multicenter study to identify lung cancer susceptibility loci by linkage analysis in high-risk lung cancer families (Anderson and Wiest) has made considerable progress, and several candidate regions have been identified. Also, a candidate susceptibility gene for mouse lung tumorigenesis has been identified on mouse Chr6 by Anderson and Wiest and collaborators at Ohio State University. Single-nucleotide polymorphisms (SNPs) are being sought in the human cytochrome P450 1A2 (*CYP1A2*) gene, so that the genotype can be correlated with phenotype (risk of cigarette smoke-induced lung cancer, head-and-neck cancer, porphyria cutanea tarda, and other diseases) (Nebert, Wiest, Grabowski). Similar studies are being carried out with the human aromatic hydrocarbon receptor (*AHR*) gene and risk of cigarette smoke-induced lung cancer and head-and-neck cancer

(Puga, Nebert, Grabowski, Foroud, Wiest). Nebert, Miles, Sallee and Grabowski are examining the role of CYP2C9, CYP2C19 and EPHX1 polymorphisms in phenytoin-induced gingival overgrowth and fetal hydantoin syndrome. Also, the mouse *Cyp1a2* gene is being replaced by a human *CYP1A2* "high-activity" allele and a *CYP1A2* "low-activity" allele (37-fold difference between the two), to be used in studying risk assessment involving aromatic amine- and dioxin-induced toxicity.

Several investigators are focusing on metal toxicity. A screen of 40 strains of *Drosophila melanogaster* has led to the discovery of a 10- to 15-fold difference between the "most resistant" (Oregon R; CT 106) and the "most sensitive" (PVM; KCA1) strains, when exposed to dietary arsenite (Cartwright). Whereas concentrations of 0.5 mM AsO_3^{-3} usually caused developmental toxicity and death, 0.5 mM AsO_3^{-3} in the food killed all females—suggesting an X-linked trait. The expressivity and penetrance of "resistance" declined with genetic crosses, indicating a polygenic inheritance (major genes plus modifier genes). Taking advantage of the *Drosophila* Genome Project having been completed, this laboratory has narrowed a major "arsenic resistance" gene to the 15C1/16F3 region (estimated as 1 million bases or slightly more) of the X chromosome; their long-term goal is to isolate by positional cloning the gene responsible for this phenotype. It is anticipated that identification of the *Drosophila* gene(s) responsible for sensitivity or resistance to As^{+++} would move quickly to discovery of the human homologous gene, which can then be screened in populations already characterized for As^{+++} toxicity (Bornschein), with help from population and statistical geneticists (Deka, Foroud). The *Cdm* gene encoding resistance to low-dose cadmium (Cd^{++})-induced testicular necrosis in the mouse has been narrowed down from more than 24 cM to less than one cM (400,000-800,000 kb) on mouse Chr 3; this work began as a funded PPP project (Nebert, Menon) and has now become a funded R01. Again, moving from the mouse *Cdm* gene, once identified, to the human orthologous gene will be relatively easy and may lead to prevention or intervention of heavy metal toxicity in humans.

Alachlor, 2,6-diethyl-N-methoxymethyl-chloracetanilide, is a prototype for several potent herbicides that have been found to cause tumors of the nasal epithelium, thyroid and stomach in rodents (and possibly humans). Metabolic activation of alachlor by one or more cytochromes P450 leads to such reactive metabolites as 2,6-diethylaniline, a *p*-hydroxynitrenium ion, and the quinonimine. CYP2A3 (equivalent to human 2A6 which is known to metabolize coumarin, warfarin and nicotine) and CYP2G1 are present in the rat nose; 2A3 exists in fetal nose and liver, but remains primarily in the nose in adult rats, whereas 2G1 is exclusively in the adult rat nose. The P450 enzymes are primarily located in the acinar portions of Bowman's glands. Feeding alachlor to rats, Genter found no changes at 1 month, but tumors (polyploid adenomas, some adenocarcinomas) appearing by 6 months, and invasive tumors beginning to appear by 10 months on an alachlor diet. Interestingly, the distribution of tumors was identical to the distribution of the above-mentioned P450 enzymes, throughout the ecto- and endo-turbinates of the rat. Enhanced DNA synthesis was found in basal cells, prior to their becoming dysplastic nodules. Mutagenesis in the Ames test was also consistent with the in vivo data. Immediate plans are to express the CYP2A3 and 2G1 cDNAs and determine which metabolites of alachlor are formed and whether they cause oxidative stress (Shertzer, Nebert).

Members of this Research Core are involved in identification of human genes that might be associated with complex diseases such as hypertension and asthma. A unique allelic variant, found in Africans and Mid-Easterners but not Northern Europeans, has been shown to have evolved in response to the hot arid climates such as the Sahara Desert and Arabia (Menon, Deka). The importance of these allelic differences in the sodium channel gene in the etiology of essential hypertension and stroke is being examined (Menon, Deka, Foroud, Broderick). The mouse aquaporin-5 (*Aqp5*) gene, which encodes the major water-channel protein in lung, was disrupted (Menon). The homozygous *Aqp5(-/-)* mouse usually dies at gestational day 15 (GD15), the time at which the *Aqp5* gene first turns on developmentally in mouse lung. AQP5 also appears to play a role in bronchial hyperresponsiveness to methacholine in asthma patients, and allelic variants in the human gene are being sought (Menon, Liggett). Response of the *Aqp5(+/+)*, *Aqp5(+/-)* and *Aqp5(-/-)* mice to H_20_2, ozone and nickel is also being studied (Menon, Leikauf). The importance of ethnic differences in pharmacogenomics-relevant genes is also being examined (Nebert, Menon). "High-blood-pressure" (HBP) and "low-blood-pressure" (LBP) mouse lines are being used to search for genes involved in stroke (Menon, Broderick), retinopathy, and renal disease. Interestingly, the HBP line has a greater number of nephrons than the LBP mouse, suggesting a possible developmental project in the near future (Menon, Potter).

Developmental toxicity is also studied in this Core. Dioxin exposure in low doses to mice causes chronic sustained oxidative stress (Shertzer, Puga, Nebert). Thalidomide and dioxin are both teratogens, are both negative in the Ames test, and therefore are not genotoxic. Thalidomide has been shown recently to effect oxidative stress in utero. Dioxin-induced teratogenesis studies, including measurements of the perturbation of homeobox (*Hox*) and various other development genes and the possible role of oxidative stress, are being studied (Potter, Nebert, Shertzer). With an SV40-immortalized metanephric mesenchymal (primordial kidney) cell line from the *Hoxa11/Hoxd11(-/-)* double-knockout mouse line, *Hoxa11* expression ("on" vs "off") was found to cause the up- or down-regulation of ~5000 genes via DNA-chip microarray analysis. One gene (α8-integrin) appears to be a "key effector gene" in *Hoxa11* signaling developmental pathways (Potter).

Knockout and transgenic mouse lines are being generated by "gene-swapping" techniques to understand the role of specific genes in responses to toxic environmental agents. Following generation of the conventional *Cyp1a2(-/-)* (Nebert, Puga, Potter) and *Cyp1a1(-/-)* (Nebert, Shertzer, Genter) knockout mouse lines in the CEG Transgenesis Core, a unified effort is now underway to develop conventional plus conditional knockouts of the *Gclc* and *Gclm* genes encoding the glutamate cysteinyl ligase catalytic and modifier subunits, respectively (Nebert, Potter, Doetschman) and the fumarylacetoacetate hydrolase (*Fah*) gene. The GCLC and GCLM proteins play a pivotal role in reduced glutathione (GSH) homeostasis and, hence, the cell's oxidative stress response. Studies to determine the extent of CYP1A2 involvement in acute liver injury, methemoglobinemia, and urinary bladder toxicity (Talaska, Shertzer, Miller, Nebert), acetaminophen-induced liver and nasal and eye toxicity (Genter, Shertzer, Miller, Nebert), halogenated hydrocarbon-induced porphyria, and numerous metabolic pathways are underway. Studies to determine the extent of CYP1A1 involvement in dioxin- and benzo[a]pyrene-induced acute liver injury, oxidative stress (Shertzer, Nebert), 7,12-dimethylbenzo[a]anthracene-induced skin inflammation and bone marrow toxicity (Shertzer, Miller, Nebert) have begun. The *Gclc(-/-)* homozygous knockout animal dies before gestational day 13, whereas the *Gclc(+/-)* heterozygote is viable and fertile. The *Gclc(+/-)* mouse exhibits a gene-dose decrease in the GCLC protein and GCL activity, but only about a 20% diminution in GSH levels—as compared with that in *Gclc(+/+)* wild-type littermates. Hence, the *Gclc(+/-)* mouse appears to be a useful genetic model for mild endogenous oxidative stress.

Example #2

Cell Signaling and Function Research Core

A. Research Core Goals and Objectives:

Signal transduction research is germane to the field of toxicology because many toxicants alter the coordinate interplay of signaling pathways through either disruptive or stimulatory mechanisms. Knowledge of signaling networks, their interplay with one another, and the biological processes they affect are useful in both predicting and defining the mode of action of toxicants. Such knowledge is also useful in the designing of strategies to modulate the consequences of toxicant exposure, and the subsequent development of acute illness/injury or disease. The overall mission of the Cell Signaling and Function (CSF) Research Core is to encourage and facilitate the interaction and collaboration of Center scientists who have interest in understanding the mediators, modulators and mechanism of signal transduction in toxicant-perturbed, normal and neoplastic cells and tissues, and to relate these signaling processes to cellular function, toxicity and human health. The broad-based goals of the CSF Research Core are to: (1) maintain a functional, interactive group of scientists who work in, or have an interest in aspects of signal transduction research; (2) develop a communal intellectual and technical expertise in the signal transduction field that remains current; (3) provide a focus for research interactions (i.e., collaborations) on topics involving environmental toxicants, and signaling and cell function; and (4) facilitate the translation of mechanistic research and insights to their application in human populations through intra- and inter-programmatic interactions and interdisciplinary collaborations.

B. Core Composition:

Core Director:
John J. Reiners, Jr., Ph.D., Core Director, Professor, Institute of Environmental Health Sciences

Core Members:
Amit Banerjee, Ph.D., Assistant Professor, Institute of Chemical Toxicology
Dharam Chopra, Ph.D., Professor, Institute of Chemical Toxicology
Cornelis J. Elferink, Ph.D., Assistant Professor, Institute of Chemical Toxicology
Lisa A. Elferink, Ph.D., Associate Professor, Department of Biological Sciences
David Kessel, Ph.D., Professor, Department of Pharmacology
Donald M. Kuhn, Ph.D., Professor, Psychiatry and Behavioral Neurosciences
Raymond R. Mattingly, Ph.D., Assistant Professor, Department of Pharmacology
Roy B. McCauley, Ph.D., Professor, Department of Pharmacology
Kamiar Moin, Ph.D., Assistant Professor, Department of Pharmacology
Frederick R. Miller, Ph.D., Professor, Barbara Ann Karmanos Cancer Institute
Michael J. McCabe, Jr., Ph.D., Assistant Professor, Institute of Chemical Toxicology
Stuart Ratner, Ph.D., Associate Professor, Barbara Ann Karmanos Cancer Institute
Barry R. Rosen, Ph.D., Professor and Chair, Department of Biochemistry
Bonnie F. Sloane, Ph.D., Professor and Chair, Department of Pharmacology
Stanley R. Terlecky, Ph.D., Assistant Professor, Department of Pharmacology
Jerrold R. Turner, M.D., Ph.D., Assistant Professor, Department of Pathology
Daniel A. Walz, Ph.D., Professor, Department of Physiology

C. Key words:

AhR (Aryl hydrocarbon Receptor), apoptosis, arsenic, cell cycle, GTPase, lysosomes, proteases, intracellular trafficking

D. Progress of the Cell Signaling and Function Research Core:

The Cell Signaling and Function (CSF) Research Core was one of the four original research programs of the Center, albeit under a different name (i.e., Signal Transduction Research Core). Although the core encompasses a large number of scientists with diverse research projects, multiple members share interests in (a) cell cycle control and apoptosis, (b) proteases, and/or (c) signaling and biological processes regulated by GTPases. During the past year focus groups in each of these areas were formally established to facilitate the exchange of information, promote collaboration, and develop a nucleus of individuals with specialized expertise. Each of the focus groups meets monthly and participation is open to all Center and non-Center members. This format has been very successful in bringing together individuals with diverse interests and perspectives.

The term ‘apoptosis’ defines a process by which cells undergo an ordered and orchestrated death. To date, the triggering of apoptosis is thought to be mediated by two pathways. The first entails processes initiated by the binding of death ligands to membrane-associated death receptors, and the subsequent activation of a specific class of proteases termed caspases. The second involves insult to mitochondria, release of cytochrome c, and the subsequent activation of caspases via a cytochrome c-activated process. Many of the therapies used to treat cancer are designed to induce apoptosis. One such therapeutic approach, termed photodynamic therapy, involves the shining of visible light on cells preloaded with a photosensitizing agent that produces toxic oxygen radicals upon illumination. Dr. David Kessel and others have shown that photosensitizing agents that localize to the mitochondria induce apoptosis via the mitochondrial pathway when illuminated. Drs. Kessel and Reiners recently reported (Photochem. Photobiol.) that a variety of cell types also undergo apoptosis following the targeted photodamage of lysosomes with lysosome specific photosensitizers. These observations raise the issue of whether there is a third pathway for the induction of apoptosis that is initiated by lysosomal proteases. This is an intriguing question since many agents that cause neuronal cell death cause lysosome damage. Furthermore, oxidant-induced apoptosis in many cell types is preceded by lysosome damage. These later observations raise the issue of whether oxidant-induced apoptosis is actually initiated by lysosomal proteases. Because of their expertise in the field of lysosome proteases, core members Drs. Sloane and Moin are collaborating with Drs. Kessel and Reiners in the study of lysosome proteases as initiators of apoptosis.

Arsenic exposure via contaminated drinking water from groundwater sources is a serious public health concern in many parts of the world. Based on epidemiological findings, exposure to arsenic has been associated with increased risk of skin, lung bladder, liver and kidney cancer. Paradoxically, despite its putative function as a

suspected carcinogen, arsenic trioxide has been used effectively in the therapeutic treatment of acute promyelocytic leukemia. The basis for the therapeutic effects of arsenic trioxide is unclear. In a recent issue of J. Exp. Pharm. Therap., Dr. Michael McCabe reported that (a) low concentrations of arsenic trioxide promote the differentiation of myelomonocytic leukemia cell line U937 and induce a G1 arrest, (b) intermediate concentrations delay transit through both the G1 and G2 phases of the cell cycle, and induce apoptosis in the cells exiting G2, and (c) high concentrations rapidly induce the death of cells irrespective of cell cycle phase. These findings suggest that low levels of arsenic trioxide, as opposed to high levels, may be clinically effective in the management of acute promyeloctic leukemia. Low concentrations of arsenic trioxide can be easily achieved in humans and would obviate complications associated with high dose therapy. These studies were performed in collaboration with several center members and used extensively the services of the Imaging and Cytometry Facility Core.

Methamphetamine is a common drug of abuse and a potent neurotoxin that targets dopamine neurons. Dr. Donald Kuhn has demonstrated that tryptophan hydroxylase (TPH) is a phenotypic marker for dopamine neurons, and inactivated both in vivo and in vitro by methamphetamine. In collaboration with Dr. Lisa Elferink data were generated implicating reactive oxygen molecules and peroxynitrite as the species responsible for the inactivation of TPH. Although peroxynitrite is a powerful oxidant, and capable of nitrating TPH, recent studies by Dr. Kuhn have shown that nitration of TPH is not the basis for the loss of activity and decreases in cellular dopamine levels. Instead, dopamine levels decrease because of the interaction of peroxynitrite with dopamine to produce the dopamine o-quinone. It is this quinone species that is probably responsible for methamphetamine-induced dopamine neuron dysfunction and damage. These findings are of considerable significance since tyrosine nitration in proteins is often interpreted as evidence of peroxynitrite action in vivo, and is used extensively as an index of toxicity in Parkinson's Disease, Multiple Sclerosis, Huntington's Disease, and Alzheimer's Disease. Dr. Kuhn's data suggest that tyrosine nitration may be an inappropriate index for assessment of neuronal damage.

The aryl hydrocarbon receptor (AhR) belongs to the basic helix-loop-helix/PAS family of transcription factors. Within this family, the AhR is the only member conditionally activated in response to ligand binding, as typified by 2,3,7,8-tetrachlorodibenz-p-dioxin (TCDD). Dr. Cornelis Elferink has recently demonstrated that the liganded AhR interacts with the retinoblastoma protein (pRb) via a LXCXE motif in the AhR protein. The association of pRb with the liganded receptor is necessary for the TCDD-induced induction of a G1 arrest in rat 5L hepatoma cells. Given the role of pRb in cell cycle progression, these findings are significant since they provide a basis for the cytostatic actions of TCDD, and represent a mechanism for the regulation of G1 cell cycle progression involving pRb that is distinct from a direct repression of E2F-mediated transcription.

The tripeptide glutathione (GSH) has a variety of physiological functions. In particular, GSH is involved in the maintenance of cellular homeostasis and protection against toxicants. Indeed, cells depleted of GSH are generally very susceptible to the killing actions of toxicants. The passaging of adherent cell lines is generally accomplished by limited exposure to the protease trypsin. Since a majority of the released cells exclude trypan blue and are viable, it is generally assumed that the conditions used for the passaging of cells are innocuous. A recent inter-core collaboration between Drs. Lash and Reiners, published in Toxicology Letters, demonstrated this assumption to be false. Specifically, these investigators showed that some cell types rapidly lose much of their intracellular GSH following short-term exposure to trypsin. GSH levels remained low for as long as 16 hours after trypsinization. During this period the cells exhibited increased sensitivity to the cytotoxic activities of oxidants. These findings are of significant practical importance. Specifically, they provide a basis for the differential responses of adherent cells and trypsin-derived cell suspensions to toxicants reported in the literature. Furthermore, they emphasize the limitations associated with the use of trypsin-derived cell suspensions.

The protease cathepsin B is normally intracellular and resides in lysosomes/endosomes. However, it is commonly found associated with the outer membrane of several tumor cells. The laboratory of Dr. Sloane has recently reported in J. Biol. Chem. and Biophys. Biochim. Acta the use of a yeast two-hybrid screening protocol to identify proteins that might be responsible for the localization of cathepsin B on tumor cell membranes. Among the proteins identified was the annexin II light chain (p11), one of the two subunits of the annexin II tetramer (AIIt). AIIt also binds other proteases [plasminogen/plasmin and tissue-type plasminogen activator (tPA)] as well as extracellular matrix proteins (collagen I and tenascin-C). Dr. Sloane and colleagues speculate

that colocalization through AIIt of the three proteases cathepsin B, plasminogen and tPA, and their substrates on the tumor cell surface may facilitate: 1) activation of precursor forms of proteases and the initiation of proteolytic cascades; and 2) selective degradation of extracellular matrix proteins. Dr. Sloane's findings are significant since recruitment of proteases to specific regions on the cell surface where potential substrates are also bound could enhance tumor cell detachment, invasion and motility. Such sites represent a potential site for therapeutic intervention in treatment of cancer patients.

The processes by which cathepsin B is targeted to the endosomes or secretory vesicles are not well understood. In collaboration with Dr. Moin the Sloane laboratory has used confocal microscopy and cathepsin B - enhanced green fluorescent protein chimeric constructs to study cathepsin B trafficking in normal and invasive human breast adenocarcinoma cell lines. Their results suggest that: 1) tumor cells have an alternative mechanism for the trafficking of cathepsin B to endosomes/lysosomes which is independent of the mannose-6-phosphate receptor pathway; and 2) the pro region of procathepsin B may contain the sorting sequence necessary for its trafficking via this pathway. These studies were recently published in Adv. Exp. Med. Biol. and relied heavily upon the services of the Imaging and Cytometry Facility Core.

The GTPase superfamily of genes encode numerous proteins involved in signal transduction and the regulation of proliferation, differentiation, apoptosis, locomotion, and vesicle trafficking. New to the Cell Signaling and Function Core is the establishment of the 'GTPase' focus group under the direction of Dr. Raymond Mattingly. As a consequence of Dr. Mattingly's initiative, Center members Tainsky and Reiners were recruited to participate in a grant applicant to the Department of Defense entitled 'Signal Transduction Targets for Pharmacological Intervention in Type I Neuro- fibromatosis.' The application was recently funded (DAMD17-00-1-0544) and entails studies in which inhibitors of the Ras/MAP kinase pathway and Rac/Rho family of small GTPases will be used to alter the phenotype of neurofibrosarcoma cells. This grant represents a melding of the expertise of three investigators with diverse backgrounds, and is serving as an excellent vehicle for the recruitment of other WSU scientists with interests in small G proteins.

Endocytosis is a process involved in nutrient acquisition, maintenance of the plasma membrane, and cellular signaling. The Rab GTPases play a critical role in the early stages of endocytosis. Studies by Dr. Lisa Elferink have shown that Rab15 co-localizes with Rab4, -5 and -11 on early endosomal membranes. The functions of the latter three Rabs have been deduced from a variety of studies; however, the role of Rab15 is unknown. Recent studies from the L. Elferink laboratory using genetically engineered variants of the Rab15 protein demonstrate that activated Rab15 reduces fluid phase and receptor-mediated endocytosis without affecting the rate of recycling from early endosomal compartments. Reciprocally, the inactive form of the protein increases the rate of early endocytosis by differentially influencing the rates of internalization and recycling from the endosomal compartment. These findings suggest that Rab15 is a key regulator of endocytic trafficking.

In addition to its goal of advancing the research programs of Center members, the Cell Signaling and Function Research Core has attempted to utilize its talents in the education of graduate students and postdoctoral fellows. During the past year members of the 'Apoptosis/Cell Cycle' and 'GTPase' focus groups, under the direction of Drs. Reiners and Mattingly, respectively, organized and offered 1-credit graduate level courses entitled 'Molecular and Cellular Determinants of Apoptosis' and 'Signal Transduction Targets in Cancer Therapy.' These courses proved to be extremely popular and well received by both students and faculty. Both courses are being offered again in 2001.

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Facility Cores

Example #1

Molecular Biology

1. Goals

The overall goal of the Molecular Biology Facility Core is to provide Center Investigators access to sophisticated and state of the art molecular techniques. Because the Center is multidisciplinary in nature, the level of support needed by different Center investigators varies widely. Consequently the level of access offered by the Molecular Biology Facility Core varies from advice on specific aspects of projects, through training on particular instruments available in the Core, to complete molecular analyses from sample preparation to the final data analysis and interpretation. The Specific Aims of the Core are: i) to provide both routine and specialized molecular biology services to Center investigators. Current services include DNA isolation, PCR, subcloning, DNA sequencing, genotyping, mutation detection and more; ii) to provide instruction and assistance to Center investigator laboratories in the use of image equipment and computer software for digital image acquisition and analysis, sequence data analysis, and sequence database searching; iii) to provide supervised access to high throughput sequencing, fragment analysis, and robotic equipment; iv) to provide expertise and consultation for a wide range of molecular biology related techniques; and v) very importantly, to identify, introduce/develop, and implement new technologies to meet the needs of Center investigators.

2. Director and Members

Jianjun Shen, Ph.D., Core Director
Director, Molecular Biology Resource Center
Science Park-Research Division
Department of Carcinogenesis
University of Texas M.D. Anderson Cancer Center

Sean Hensly, B.S., Senior Research Assistant
Angela Tanzillo-Swarts, B.S., Research Assistant II
Lisa Sherry, B.S., Research Assistant I

3. Facilities and Equipment

The Molecular Biology Facility Core occupies two offices, one room for the Image Analysis Facility, one wet laboratory, and an automation and high throughput laboratory in Lab 3. It has an ABI PRISM 377 DNA sequencer, a Beckman Biomek 2000 Laboratory Automation Workstation, three Gel Documentation Systems, a PhosphorImager Storm 820, a FluorImager SI, an InstantImager, and a Kodak Digital Science™ Image Station 440CF.

4. Usage and Benefits of the Facility Core

Virtually all investigations into the mechanisms involved in environmental toxicology in general and carcinogenesis in particular have benefited from the tremendous progress that has been made in the molecular understanding of disease processes and environmental interactions. As a result, most projects of Center members involve Molecular Biology at some level. Thus, the ability of the Center to provide support for a variety of molecular techniques is a critical factor in the success of the Center.

Many Center investigators have been able to have samples analyzed in the Molecular Biology Facility Core that could not have been done in their own laboratories, either because of the lack of experienced personnel or the necessary analytical equipment. The Molecular Biology Facility Core has provided services ranging from genotyping, manual and automated DNA sequencing, to mutation analysis.

The Molecular Biology Facility Core has also become a centralized high tech instrumentation center for image acquisition and analysis, as well as DNA sequence data analysis. Simply having access to the Molecular Biology Facility Core facilities has promoted collaborations among Center investigators within and between Research Cores, as well as interactions between Research Core members and the Molecular Biology Facility Core.

The Molecular Biology Facility Core has directly contributed to research and development of new and advanced methods for sequencing, mutation detection, genotyping, and proteomics for Center investigators. One such example is the centralized sequencing facility that contains an ABI 377 Automated Sequencer. This facility has dramatically improved throughput in this Facility Core and eliminated the need for individual laboratories to set up manual sequencing equipment. Since 2000, it has introduced a whole spectrum of sequencing chemistries to routinely sequence normal and difficult (such as AT-rich or GC-rich) DNA. Center investigators with high throughput needs have direct access to the instrument.

Many Center investigators are utilizing animal models for their research. In particular, CRED investigators now maintain increased numbers of transgenic mice, and in virtually all cases genotyping of colonies is done by molecular methods. Based on a survey conducted in September 1998 and built upon its success in 1999, the Molecular Biology Facility Core has continued to improve and expand the existing genotyping services. In year 2000 alone, the Molecular Biology Facility Core has implemented 13 new PCR-based genotyping methods such as those for Eker rat, IGF, APRT, and RAS transgenic mice. These improvements have directly benefited Center members, Drs. Fuchs-Young, Johnson, Li, Richie, Walker, and Wong.

Since the beginning of year 2000, the Molecular Biology Facility Core has started to move ahead into proteomics, particular in 2D gel electrophoresis. A Protean IEF Cell (Bio-Rad) was purchased for the isoeletric focusing procedure. The Core has so far established procedures such as protein sample preparations from various tissues and cell cultures, isoeletric focusing, SDS-PAGE, in-gel trypsin digestion, and Western blotting. Trypsin digested protein samples will be sent to the Analytical Instrumentation Facility Core of the Center at UT-Austin for protein sequencing and other related analysis. This will facilitate Center members using that Facility Core. Two-D gel electrophoresis is also a perfect complementation to functional genomics. It thus enhances interactions among the Facility Cores of Molecular Biology, Analytical Instrumentation, and Functional Genomics. More importantly, the introduction of this technology will further strength the Core to serve as a focus for interactions among Center members. The Molecular Biology Facility Core Director, Dr. Shen, has been collaborating with Drs. Bedford, MacLeod, and Wong utilizing 2D gel electrophoresis for their respective research projects. Other Center members, Drs. Aldaz, Conti, DiGiovanni and Tang, have expressed their intent to utilize the technology.

The Molecular Biology Facility Core held a workshop in February 2000. More than 25 people (including Center members, postdoctoral fellows, graduate students, and research assistants) from UT/MD Anderson Cancer Center in Houston, Science Park – Research Division in Smithville and UT-Austin attended the workshop. They were made further aware of the Molecular Biology Facility Core's current services, especially many newly developed and/or implemented genotyping services, and newly introduced automated DNA sequencing chemistries. They were also trained in the use of many databases for their DNA and protein analysis via internet.

Taken together, the Molecular Biology Facility Core has provided Center investigators a wide-range of molecular biology related services, developed and introduced new methods and techniques, and consulted and assisted in image acquisition and analysis.

Example #2

Cell Markers Facility Core (CM-FSC)

1. Description / Objectives

The purpose of the Cell Markers Facilities and Service Core (CM-FSC) is to provide analysis of chemical and molecular markers of cellular and animal responses to toxicants.

Treatment of animals in vivo or cells in vitro with chemicals produces a multiplicity of end points. Standardized quantitative evaluations of the effects of toxicants on animals and *in vitro* models is an essential part of toxicology and is essential for comparisons of research being done in different laboratories. The production of a key toxicant metabolite may be critical to understanding the mechanism of action of the toxicant. Formation of macromolecular adducts with a reactive toxicant intermediate can initiate changes in the normal, molecular signaling processes. By determining the changes in the levels and/or modification of specific enzymes, hormones, other signaling molecules and signaling proteins (including kinases, receptors and cell cycle regulators) new insight is possible on several fronts. A mechanism of toxicant action may be suggested. Alternatively, the changes could provide the means for quantitatively evaluating the progress of the toxicity process and comparing the relative potencies and effectiveness of different toxicants. These endpoints can be collectively viewed as cell markers of toxicity. Quantitative measurement of such cell markers frequently provides an index of toxicant exposure and activity.

The Core's emphasis on bioanalytical methodology is in the following areas:

* Structural identification of macromolecular adducts formed from toxicants (In the future this will be limited to immune and post-labeling methods with the establishment of a Mass Spectrometry FSC;

* Qualitative and quantitative analyses of the levels and modification of proteins characteristic of cell signaling pathways or other cellular processes in response to toxicants;

* Total analysis of cell protein expression following cell culture or developmental changes. This includes exposure to hormones and toxicants. This can also be coupled with isolation of proteins for analysis of sequence and modification by mass spectrometry.

2. Members:

Cell Markers FSC Leaders / Staff

Terry D. Oberley, M.D., Ph.D., Prof	Facility Core Co-Leader; Department of Pathology
Santhanam Swaminathan, Ph.D.,Sr. Scientist	Facility Core Co-Leader; Department of Pharmacology
Melissa Burger	Associate Research Specialist, Department of Pharmacology
Faye Bruggink	Research Specialist, Department of Pharmacology

Advisory Committee:

Thaddeus Golos, Ph.D., Assoc. Prof.	RC1	Department of Obstetrics and Gynecology
Elaine Alarid, Ph.D., Asst. Prof	RC2	Department of Physiology
Jeffrey Johnson, Ph.D., Asst. Prof	RC3	Division of Pharmaceutical Sciences

3. Equipment and Facilities

Instrumentation for the Cell markers FSC is currently located in Dr. Swaminathan's laboratory located in room 2695 of the Medical Sciences Center. These facilities are designated for relocation to the newly assigned laboratories (Room 184) located at the Enzyme Institute. This move will relieve crowding and facilitate expanding demand for the Image Station services. The specialized instruments/equipment include: 1) a Kodak ImageStation 440CF - this instrument provides a complete digital imaging system used for detection of proteins and nucleic acids in gels, membrane blots and X-ray films; 2) a Protean IEF Cell System - the IEF Cell system accomplishes isoelectric focussing of proteins on IPG (Immobilized pH Gradient) ReadyStrips followed by SDS-PAGE electrophoresis; and 3) PDQuest Software – a sophisticated software package for analyzing and databasing of 2-D gels of proteins. This

system permits image acquisition with the Kodak Image Station 440CF and permits comparative analyses of 100 gel profiles at a time.

4. Usage and Benefit

The CM-FSC provides analytical measurements for a variety of toxicological endpoints. The particular methods offered in this FSC are based on Investigator input and meet the following three needs: a) where the Investigator does not have experience with a particular technique (e.g. 2-D gel electrophoresis); b) where additional probing of expensive protein samples or electrophoresis membranes would be beneficial to the Investigator and possibly open up new mechanistic insights and collaborations; and c) where the FSC can provide a cost effective alternative to use of a routine assay in the Investigator's laboratory. For all assays, standardization of a procedure used by multiple Investigators is an additional benefit. The generation of labeled probes that can be used for samples from several laboratories may be very cost effective.

The combination of 2D-gel electrophoresis and mass spectrometric analysis of individual protein spots on the gels has opened up the field of Proteomics, which seeks to provide a comprehensive analysis of the proteins expressed in a cell under a given set of conditions. The most powerful MS approaches can now provide detailed information on the sites of post-translational modification of protein. This type of analysis provides great opportunities for characterizing the mechanism of action of toxic chemicals. The CM-FSC has been working towards this analytical goal. The CM-FSC will continue the protein separation component of this work and then pass the MS component to the new MS-FSC.

Depending on the nature of the endpoint and the capabilities of the Investigator, the CM-FSC provides various levels of support, ranging from consultation and training in sample preparation to coordination with existing campus facilities, to actual analytical services on a for fee basis. CM-FSC staff operate the Kodak Image Station and 2-D electrophoresis systems and train laboratory personnel to use these instruments. Cost of the common reagents for chemiluminescent and fluorescent image analyses and for use of the Kodak Image Station analyses is assumed by the CM-FSC. Stocks of certain secondary antibodies are maintained to expedite analyses for Center members. In addition, initial consultation on experimental procedures and instruction on data collection and analyses are supported through the Center grant as a benefit to members and to encourage new and expanded usage. The CM-FSC has also participated in cost sharing of charges to Center investigators for the services provided by the UW-Biotech Center for mass spectrometry or the UW-NMR facility for nuclear magnetic resonance analyses of samples when coordinated through the CM-FSC.

Cell Markers FSC Contributions to Center Investigators' Research

Measurement of Adducts

** Dr. Tom Moll from Dr. Elfarra's laboratory worked through the CM-FSC to get mass spectral analyses of hemoglobin adducts formed by butadiene epoxide. These analyses are critical to a project investigating the mechanisms of toxicity of butadiene and related conjugated dienes. ** Dr. Swaminathan's lab has developed ^{32}P-postlabeling methods for qualitative and quantitative analyses of DNA adducts generated from the human bladder carcinogen, 4-aminobiphenyl and related environmental toxicants. The structural identities of these adducts were established by conventional spectroscopic analyses involving mass spectroscopy and proton NMR. These bioanalytical methods will be useful in the development of markers of exposure to environmental toxicants. The functional impact of these bulky DNA adducts on mutagenesis and carcinogenesis are currently under investigation.

Kodak Image Station

** Dr. Colin Jefcoate's laboratory has used the Image Station for the analysis of cytochrome P450s, AhR receptors, cyclins, p53, CDK inhibitors, and PPAR following exposure of mouse embryo fibroblast cells and human breast epithelial cell cultures to TCDD or polycyclic aromatic hydrocarbons. ** Dr. S. Swaminathan's laboratory has utilized the Image Station for the analysis of cell cycle (cyclins, pRb) and DNA repair proteins, such as p53, MDM2, GADD45 and WAF1), in both mouse embryo fibroblast cell cultures and bladder transitional cell carcinoma (TCC) lines. ** Dr. Schuler's lab has applied the Image Station to investigate prolactin-mediated alterations in cyclins and p21 and their effect on cell cycle progression of human mammary epithelia. ** Dr. Oberley uses the service to

analyze for oxidative damage with antibodies to oxidative damage products, including 4-hydroxy-2-nonenal protein adducts, protein carbonyls, and nitrotyrosine. Tissues studied will include rat kidney after treatment with iron nitrilotriacetate or heat stress and mouse heart tissue after treatment with adriamycin. ** Dr. Verma's group uses the Image Station facility for quantitation of protein kinase C (PKC) following treatment with tumor promoters. They have used the equipment for characterization of various isoforms of PKC. ** Dr. Barnes' laboratory used the Image Station facility to examine the effects of mercury contamination on adipocyte differentiation and function. Specifically, they have examined the Hg-induced changes in cell signaling pathways and stress proteins (Bcl, bax, p21, p53). ** Dr. Czuprynski's group studies the differences in protein expression between treated and untreated preB and stromal cells during DMBA-induced apoptosis. Using mass spectrometric analysis (MALDI-TOF) they plan to determine some novel proteins involved in apoptosis. ** Dr. Johnson's laboratory has been investigating the role of oxidative stress in neuronal diseases and anticipates using the CM-FSC for Western analyses. ** The laboratory of Dr. Susan Smith used the imaging station to quantify western blots. The results indicated increased protein levels of HIF1-alpha, a hypoxia response protein in the hearts of AhR knock-out mice. The use of the Imager greatly enhanced the ability to quantify these protein levels when compared to traditional film.

2-D gel electrophoresis
** Dr. Swaminathan's lab is investigating the post-translational phosphorylation of p53 and its role in transcriptional regulation of proteins involved in repair of DNA damage caused by environmental agents. Towards this goal they have been using the 2-D gel electrophoresis system to characterize the various phosphorylated forms of p53. ** Dr. Jefcoate's laboratory has used the CM-FSC 2-D gel electrophoresis system to investigate the role STAR proteins and their post-translational modifications during steroidogenesis. ** Dr. Mulcahy's group have applied the 2-D gel electrophoresis equipment and the technique to examine the phosphorylation of Nrf2, a bzip factor involved in transcriptional up-regulation of phase II enzymes through Antioxidant Response Element (ARE). ** Dr. Wilding's laboratory has utilized the 2-D electrophoresis system to identify changes in proteins that are coincident with androgen exposure and the generation of reactive oxygen species in prostate tissues. Secondly, they propose to identify the proteins that bind with the AP-1 site, the modulation of these proteins by environmental agents and their impact on prostate cancer.

Training of Investigator Staff and Students
CM-FCS conducts several workshops to increase the awareness of the facility and to train students and the staff affiliated with the EHS Center. Additional presentations are made during the poster sessions at the annual Center retreat.

Example #3

Laboratory Supplies and Services Facility Core:
Objectives: The objectives of the Laboratory Supplies and Services Facility Core are to provide NIEHS Center researchers with several basic research and laboratory needs including

high quality discounted tissue culture and bacteriological media, buffers, and serum
an on-site commercial supply of molecular biology enzymes and reagents, and
cost efficient dishwashing, autoclaving and general laboratory maintenance services.

This very active purchasing and service facility core provides daily research support to NIEHS Center investigators who employ cell culture and molecular biology in their research on environmental health issues. In addition to the significant cost savings that are realized for daily purchases of tissue culture media, serum, and molecular biology enzymes and reagents, the facility also provides inter-laboratory consistency, quality control, and reduced personnel requirements by shifting the performance of dishwashing, autoclaving, and general laboratory maintenance tasks to the facility technician.

Members:

Catherine B. Klein, Ph.D., Core Director, Assistant Professor of Environmental Medicine
JoAnn Rapala, Media Preparation and Laboratory Maintenance Technician
Linda Tomlinson, Dishwashing and Laboratory Maintenance Technician
Joanna Lesczcyneska, Manager, Molecular Biology Freeezer Program

Facilities and Equipment:

The Molecular and Cell Biology Supplies and Services Facility Core manages a Roche Molecular Biology on-site stock room which is equipped with alarmed refrigerator and freezer storage space. In addition, the facility has arranged favorable purchasing contracts with other molecular biology suppliers to acquire enzymes and other reagents at discounted costs, with free overnight deliveries. The facility core maintains and schedules the shared use of general laboratory glassware dishwashers and large capacity laboratory equipment autoclaves. Other facilities and equipment used by this facility include an outfitted tissue culture room with a Millipore water purification system, laminar flow hoods and incubators that can be used for the preparation and sterility testing of media and buffers; a walk-in cold room for media storage; a liquid nitrogen tank for long term storage of available cell lines; and a -20^{o}C freezer for storage of fetal bovine serum. In 2000, the facility core updated its water purification capabilities with the purchase of a new Millipore Milli-Q Biocel/Rios 8 system.

Usage and Benefits: The in-house availability of freshly prepared tissue culture media and serum, as well as molecular biology enzymes and reagents, provides investigators with rapid access to these items at very substantial cost savings. This facility has prepared several thousand liters of tissue culture media and buffers for NIEHS investigators since 1994, and has shown steady increases in usage since that time. In support of increasing tissue culture based research at the Center, the in-house availability of US origin fetal bovine serum (including heat inactivated and dialyzed serum) obtained at a favorable discounted bulk purchase price had been a valuable resource to many investigators. Consultation is provided to investigators regarding preparation of specialized tissue culture media, the identification of less expensive or alternate media substitutes, as well as training in basic tissue culture protocols. Centralized access to dishwashing and glassware sterilizing equipment is coordinated by the facility technician who also provides scheduled general laboratory maintenance and dishwashing services upon request. Molecular biology projects continue to be supported by immediate access to enzymes and reagents from the on-site commercial stock room. The facility typically processes several hundred orders per year for molecular biology enzymes, reaction kits, reagents, and other molecular biology supplies. Molecular biology supplies not included in the on-site inventory are obtained the next day from three different suppliers, with a waiver of shipping costs. This affords NIEHS researchers with timely access to the most current molecular biology supplies available. Overall, this multifaceted facility core provides important tissue culture and molecular biology research support to all NIEHS Center investigators, while at the same time significantly reducing research costs and purchasing delays.

In 2000, the Laboratory Supplies and Services Facility Core provided tissue culture media/serum and/or molecular biology enzymes and reagents to 18 funded investigators, in support of over 30 peer-reviewed funded grants.

Community Outreach and Education Program

Example #1

Goals and Theme: The focus of research at the NIEHS Center for Environmental Health Sciences at the University of California, Berkeley is the relationship between DNA damage and disease; the Center's objective is to discover ways to prevent or mitigate such damage. The focus of the Center's COEP is to translate the basic scientific discoveries made by Center researchers into a form accessible to health care providers, community organizations, teachers, and K-12 students. A particular focus of the COEP is programs involving nutrition and health. A key objective of the COEP is to increase awareness of the importance of healthy diets in preventing cancer and heart disease among members of the community, especially socioeconomically disadvantaged populations. The COEP often targets its efforts to those people who are at the hubs of disseminating nutrition information in the community. Pursuant to this goal, the COEP provides nutrition education curriculum packages, computer-based learning materials, seminars, and workshops to organizations responsible for nutrition education in public schools in California, WIC and Food Stamp Programs, as well as to health care professionals and organizations. The COEP also strives to collaborate with community groups working on environmental and public health issues. It is the goal of the COEP to work with such groups and assist them by promoting the transfer of useful scientific information when they want a clear understanding of scientific issues, including the actual nature of threats posed by specific chemicals or foods, and the protective effects of particular foods and micronutrients such as anti-oxidants.

Members:

Patricia Wakimoto, DrPH, RD, COEP Director
Program Officer, position being filled

Collaborations

- University of Southern California Cancer Genetics Unit
- UC Cooperative Extension, the outreach arm of the University of California
- Expanded Food and Nutrition Education Program (EFNEP) and the Food Stamp Nutrition Education Program (FSNEP) provides nutrition education to low-income families, food stamp program participants and teachers in K-12 public schools
- Adelante, a not-for-profit organization which serves primarily the Latino population in the northern California Bay Area. The organization provides adult education, vocational training and referrals to other community resources.
- West Berkeley Family Practice Clinic, a provider of primary care and social services to low-income people of all ages
- Nuestra Esperanza, a not-for-profit organization, which operates a multi-cultural activity center. Nuestra Esperanza's mission is to promote the emotional, spiritual, social, educational, and physical well-being of Latino families, individuals and others separated from the community by language and cultural barriers. Nuestra Esperanza is committed to meeting the needs of all individuals who lack support, with specific attention being given to those who have traditionally not been able to access services.

Highlights

Diet and Disease Education in the Latino Community
The Center implemented an outreach and education program which targeted the Latino population. Last year the Center was instrumental in the development and testing of the culturally appropriate nutrition education

tools for the program. This year, the project provided dietary assessments and nutrition education to several hundred Latinos and their families. Local community sites included Adelante, Nuestra Esperanza, a Berkeley Family Practice Clinic, and a local job fair. Two brief self-assessment tools were used to analyze the diets of the participants and provide immediate feedback to individuals regarding their individual intake of fruits and vegetables and fat. Handouts with practical tips for increasing fruits and vegetables and decreasing dietary fat were developed specifically for the project. Additional materials were given out, depending on their level of interest by individuals and families in receiving more information. Participants were found to be very receptive to the assessment tools and the written materials; a high level of feedback was received.

Nutrition Update Conference
The NIEHS Berkeley Center sponsored the Nutrition Update conference on October 12, 2000 at the Clark Kerr Campus in Berkeley. Center scientists including Director Bruce Ames and Dr. Len Bjeldanes presented an update on research findings to Cooperative Extension Nutrition personnel and Nutrition, Family and Consumer Science Advisors for the counties of California. The sixty attendees included Nutrition Advisors, Nutrition Education Assistants, and Programs Representatives representing statewide and regional agencies throughout California. The paraprofessional, Nutrition Advisors and Nutrition Education Assistants provide direct nutrition education to the low income population throughout California. The Director of Outreach presented "Tools for Nutrition Education and Outreach". Participants were provided with interactive nutrition education CD ROMs (developed by Center scientist, Gladys Block) as well as other assessment tools.

Folic Acid Education Project
This NIEHS Berkeley Center sponsored teacher training workshops as a part of the Folic Acid Education Project, which was conducted by the University of Southern California Cancer Genetics Unit. The project's goal was to increase awareness and consumption of folic acid in high school students. Three curriculum development and evaluation workshops were conducted for teacher/student teams from five high schools in the East Los Angeles area. The core curriculum was developed and field tested by 100 students at a local nonparticipating high school. The five participating schools received the completed curricular materials. The students received the curriculum in the classroom and completed pre- and post-tests. A poster of the project will be presented at the March of Dimes meeting, "Preventing Birth Defects and Infant Mortality" in January 2001.

Materials and Publications.

Nutrition education handouts were developed in both English and Spanish language, which offer specifics tips to increase fruit and vegetable intake and decreasing fat intake.

The development of the two nutrition education tools, the "Fruit and Vegetable Screener" and "Fat Screener", was completed. These brief questionnaires are used as self-assessment tools to evaluate fruit and vegetable and fat intakes. The food items were based on national data on the Mexican and Mexican American population. The questionnaires were pilot tested last year and administered on a larger scale this year.

Example #2

Objectives: The COEP Objectives mirror those of the NIEHS Marine and Fresh Water Biomedical Sciences Center:

1. To foster collaborative investigations in the two Center target research areas:
   - **Marine & Fresh Water Toxins and Human Disease**
   - **Marine & Fresh Water Models of Human Health**
2. To educate and serve as a resource concerning the human health and environmental impacts of the Marine and Fresh Water Toxins and the importance of Marine and Fresh Water Models for our target groups: health care providers and patients (as well as the scientific community, media and the general public).

The COEP activities of the NIEHS Center have expanded in several directions over the past 10 years, thanks to the scientific and monetary support of the NIEHS Center as well as the Rosenstiel School of Marine and Atmospheric Sciences, the University of Miami School of Medicine, the University of Miami School of Arts and Sciences, the Florida Poison Information Center, the Florida Dept of Health, the Florida Harmful Algal Bloom Taskforce, Florida International University, and various Florida healthcare providers and public health practitioners. The major areas of development of COEP have been:

- **Harmful Algal Bloom Surveillance**
- **Harmful Algal Bloom Education and Outreach targeted at patients and Health Care Providers**
- **K-12 Education: the AMBIENT Project and INSTAR**

In addition, the COEP at the NIEHS Center has been the source of significant **new Research** into the Human Health Effects of Harmful Algal Blooms.

1) **Marine & Fresh Water Toxin Hotlines (888-232-8635)**: The NIEHS Center created and contributes to the Marine and Fresh Water Toxin Hotline of the South Florida Poison Information Center. The South Florida Poison Information Center receives calls on marine and Fresh Water illnesses from all over Florida, the US and internationally. It is a toll free 24 hour 365 day/year multi-lingual (English, Spanish and Haitian Creole with availability of 125 other translated languages) hotline. It provides diagnostic, treatment and educational information, and will report officially cases of reportable diseases (such as Ciguatera, PSP, Scombroid, tetrodotoxin poisoning, and NSP) to the Florida Dept of Health. Additional informational follow-up is performed by referral to NIEHS Center personnel. In addition, this Hotline is known as the Marine and Fresh Water Toxin Disease Resource for the national and international Poison Control Network. This Hotline serves not only as an informational resource, but also to begin the process of case surveillance for the highly under-reported Marine and Fresh Water Toxin Diseases. A packet of general information on the Marine & Fresh Water Toxin Diseases is sent to callers at their request; it also posted on the Center Web site. Referrals are made for clinical treatment, laboratory testing, surveillance recommendations, case reporting, and to the Speakers Bureau.

2) **Local and National Collaborations & Educational Activities**: The NIEHS Center Website (http://www.rsmas.miami.edu/groups/niehs.html) provides one of the few scientifically valid websites focused on the human (not just environmental) health effects of the Harmful Algal Blooms. In addition to administrative and scientific information concerning the NIEHS Center, there are patient and healthcare provider targeted downloadable information concerning the diagnosis, treatment, prognosis and epidemiology of the marine and Fresh Water toxins and human health. In addition, the TOXMASTER email site linked to the NIEHS Center Website has provided a venue for patients, healthcare providers, as well as other scientists, reporters, lawyers, and the general public, to anonymously ask questions concerning possible human health effects of the harmful algal blooms. The COEP receives at least 1-3 queries/day from Florida, the US, the Caribbean, and internationally as well as regularly providing packets of educational materials (see below).

A VideoConference entitled “Harmful Algal Blooms in Florida” (June 1999) was developed by Center Investigators with support from the CDC and Florida Dept of Health, and broadcast to Florida physicians and health department personnel. A video, annotated slides and educational materials were developed for distribution both locally and regionally.

3) **NIEHS k-12 funded AMBIENT Project (Atmospheric and Marine-Based Interdisciplinary Environmental health science Training)**: Miami-Dade County is home to more than 2.1 million

people. Ethnic diversity is extensive, with a population that is 52% Hispanic, 34% African American, 13% White, and 1% American Indian/Asian/Other. As with any community of this complexity, there are significant environmental health issues of concern to the community and government. The Miami-Dade County Public Schools is the 4th largest district in the country with more than 350,000 students, more than 93,000 of which are in grades 9-12. There is significant need within the public high school system to involve students with research scientists and members of the community in an interdisciplinary approach to learning about local environmental health science issues.

The recently NIEHS funded AMBIENT Project (Atmospheric and Marine-Based Interdisciplinary Environmental health science Training) is a systemic approach to environmental health science education. Focused around the four environmental themes of air, water, soil and food, a health-science problem-based learning approach will be delivered by trained teachers to the ethnically diverse population of high school students in Miami-Dade County. The teachers will work together to enhance understanding of environmental and ethical issues through a hands-on summer workshop with research scientists from the University of Miami, Florida International University, and County Department of Health. Best practices from existing environmental curriculum materials will be assembled for use in the training. An important emphasis of the project will be to provide team teaching strategies for incorporating interdisciplinary activities into the large classes of more than 35 students at the high schools.

The project is modeled after three highly successful environmental teacher training models, GLOBE, INSTAR and the SECME Summer Institutes, and draws the best from each. Classroom activities and assessment tools will be incorporated by the Center for Educational Technologies at Wheeling Jesuit University into a problem-based learning Web site similar to the NASA Exploring the Environment series. An outside Evaluator will provide formative and summative assessment of the project. This project addresses the need defined by Priority 8.2 of Healthy People 2000: Educational and Community-Based Programs, which is to increase high school completion rates to 90 percent, especially with regard to Hispanic and Black American students.

The Principal Investigator is Dr Patrick Walsh while other Center Investigators will participate at Environmental Health Scientists in the development of curriculum and course teaching. Dr Lisa Pitman, a Miami Dade County Environmental Science High School Teacher, is the Study Coordinator of AMBIENT.

4) **COEP generated Research:** Prior to the development of the University of Miami NIEHS Center, there had been very little research, particularly from an epidemiologic and clinical perspective, of the human health effects of the Harmful Algal Blooms. As a result, the activities of NIEHS COEP have been a source of considerable new research in this area, including:

- The Pilot Study of Aerosolized Red Tides and their Effects on Human Health
- Neuropsychological Effects of Ciguatera Fish Poisoning
- The Pilot Study of Occupational Harmful Algal Blooms
- Ciguatera Fish Poisoning Reporting by physicians in an endemic area
- Reported Ciguatera Cases in South Florida using Establishment of a Marine Seafood Toxin Disease Hotline
- Outreach and Education through the Establishment of a Marine Seafood Toxin Disease Hotline
- GIS and the Epidemiology of the Marine Toxin Diseases (NIEHS Shannon Award)
    - Blue Green Algal Toxins, Surface Drinking Water, and Hepatocellular Carcinoma using GIS
    - Geographic Information Systems (GIS) and Endemic Ciguatera Fish Poisoning

Members:
Lora E Fleming MD PhD MPH, Associate Professor, Outreach Director
Lisa Pitman Ed (Miami Dade County Public School teacher) Study Coordinator of the AMBIENT Program
Richard Weisman PharmD (Director South Florida Poison Control & Associate Professor, Dept of Pediatrics)
Dan Baden PhD, Adjunct Center Investigator and Former Center Director
Donna Blythe MD, Adjunct Assistant Professor, Dept of Medicine)
Helga Dienes MA (Graduate Student)
Center Investigators

Collaborations: Recent research proposals and other activities have involved collaboration with researchers regionally, nationally and internationally as well as with state and Federal agency personnel. Specific activities have ranged from Clinician and public education to seafood testing to collaborative grants and Investigator training.

- *State*: Florida Depart of Environmental Protection; South Florida Water Management District; Health Depts of Florida, Texas, Louisiana, California, Mississippi, Alabama, NC, Maryland, Delaware, Virginia
- *Federal*: NIEHS, Centers for Diseases Control and Prevention (CDC), NOAA/NMFS: interagency Working Group on HAB; NCRR (NIH): The Aplysia Facility; FDA: Seafood Safety Group; SeaGrant (Florida, Puerto Rico); NSF, ECOHAB, Interagency Taskforce on Coastal Issues
- *Universities*: Duke University, University of Tennessee, University of Maryland Center of Marine Biotechnology, University of Washington, Johns Hopkins School of Medicine, University of North Carolina at Wilmington, Harvard University, University of Miami Sylvester Cancer Center, Florida International University, Old Dominion University, Virginia Marine Institute
- *Other*: Mote Marine Laboratory, Miami Seaquarium, National Neurofibromatosis Foundation, South Florida Poison Control, South Florida Aquarium Society, Woods Hole Oceanographic Institute, North Carolina Water Resources Research Institute, Lovelace Respiratory Research Institute, Harbor Branch Oceanographic Institute, Miami Exhibits Inc, American College of Occupational and Environmental Medicine, Society of Behavioral Toxicology, Argentinean National Agency for Food and Agriculture, Gordon Research Conferences, Pan-American Institute of Food and Zoonosis Protection, National Research Council Canada Food Toxins, Pan American Health Organization, World Health Organization, Bermuda Biological Research Station
- *NIEHS Environmental Centers*: Duke University, AUEHSC Center Consortium, Zebra Fish Consortium, UMDMJ & University of Maryland (Pfiesteria)

Additional Highlights (sponsored in part by COEP, in part by other sources):
- Center Investigators presented Outreach activities at International Society of Environmental Epidemiology, the NIEHS Center Directors Meeting and CDC Pfiesteria Conference (2000)
- Center 2000 Seminars: Dr. David Carlson, Penn State University, *Steroid Hormone Superfamily Receptors: The Crossroads of Endogenous and Xenobiotic Signaling;*Dr. Lee Ann Woodward, Fish and Wildlife Service,*Tales of the Toxic Pacific;*Dr. Kevin Kleinow, Louisiana State University, *Food Chain Transfer of Xenobiotics: Bioavailability and Biotransformations in the Catfish Intestine;*Dr. Martin Grosell, McMaster University, *Copper Homeostasis and Toxicity in Fish;*Dr. Izhar Khan, University of Texas, *A Teleost Model of Neuroendocrine Toxicology;*Dr. Marc Slattery, University of Mississippi, ,*Surviving Anoxia with the Brain Turned On*; Dr. Patricia Schulte, Department of Biology, University of Waterloo, *Gene regulation,*

environmental stress and evolution; Dr. Francois Lallier, IFREMER, Roscoff, France, *How to be a perfect host: sulfide and carbon dioxide physiology in the hydrothermal vent tubeworm, Riftia pachyptila;* Dr. Kenneth B. Storey, Carleton University, Department of Biology, Ontario, Canada, *Metabolic Depression: How Animals Live Without Oxygen;* Dr. Vance Trudeau, Department of Biology, University of Ottawa, ***Neural Regulation of Reproduction in Fish;***

- Center Investigators participated in "Oceans and Human Health Course" for Masters students sponsored by Burroughs Welcome and the University of North Carolina at Wilmington at the Bermuda Marine Biological Research Station (2000);
- Dr. Jelle Atema, Boston University, First Annual Distinguished Lecture in Marine Neuroscience, Sponsored by the Neuroscience Program and the Marine and Freshwater Biomedical Sciences Center of the University of Miami,*Tracking Underwater Odor Sources: Lobster Neurobiology, Behavior and Robots* (2000)*;*
- Center Investigators participated in a Media Workshop Day entitled "Remedies from the Sea" in conjunction with the release of NAS Report on "The Oceans' Role in Human Health" (1999);
- Center Investigators developed and participated in interdisciplinary, multi-institutional Research and Outreach response to the human health effects of aerosolized Florida Red Tide (Brevetoxin) (1999);
- Center Director presented at the Annual Meeting of the Canadian Society of Zoologists in Ottawa, Ontario and at the 5th International Congress of Comparative Physiology and Biochemistry in Calgary;
- Center Investigators presented and participated in "Oceans and Human Health" Symposium sponsored by NIEHS and IOC at the Bermuda Marine Biological Research Station (1999);
- A VideoConference "Harmful Algal Blooms in Florida" was developed by Center Investigators with support from the CDC and Florida Dept of Health, and broadcast to Florida physicians and health department personnel (1999);
- Center Investigators hosted the First Annual Meeting of the NCRR Resource Directors attended by the Directors and staff of the 25 NIH NCRR Resource Centers;
- Center Investigators present at AAAS Symposium on Marine Toxin Diseases (1999);
- Center Investigators collaborate on harmful algal bloom investigations for the State of Florida with the Florida Depts of Health and Environmental Protection and South Florida Poison Control Center, including an epidemiologic study of DEP workers exposed to fish kills in estuarine waters and a clinical study of individuals exposed to estuarine water (1998-99);
- Center Investigators initiate the use of geographic information systems (GIS) to study marine toxin issues, and combine human, biological and oceanographic data (1997-99);
- Center Investigators participate as members and scientific advisors to the Florida Dept of Environmental Protection Harmful Algal Bloom Taskforce, including hosting a meeting with a NIEHS Center Tour (1998-99);
- For the Year of the Ocean (May-Sept 1998), the Center was the official Coordinator for the NIEHS scientific exhibitions at the 1998 World Expo "Oceans and Human Health" US Pavilion (Lisbon, Portugal) in collaboration with other NIEHS Centers, NOAA, the US Navy, NAS, and various States. At least 5,000 people attended the exhibit each day from all over the world. The displays included an Aplysia touch tank, interactive computer programs from NIEHS Centers, models of nerve channels and Aplysia, and a touchable iceberg. A more complete description of these scientific exhibits has been posted on the Center Website;

Materials and/or Publications:

In addition to the NIEHS Center Website, other materials have been created over the past 10 years of the NIEHS Center by Center Members which are distributed free of charge on request via website, phone, fax, regular mail, and email:

Baden D, Fleming LE, Bean JA. **Marine Toxins**. In: Handbook of Clinical Neurology: Intoxications of the Nervous System Part II. Natural Toxins and Drugs. FA deWolff (Ed). Amsterdam: Elsevier Press, 1995;21(65):141-175.

Blythe D, Fleming LE, Ayyar DR, Baden D, De Sylva D, Shrank K. **Mannitol Treatment for Acute and Chronic Ciguatera fish Poisoning.** Memoirs Queensland Museum 1994;34:465-470.

Blythe DG, De Sylva DP, Fleming LE, Ayyar RA, Baden DG, Schrank K. **Clinical Experience with IV Mannitol in the Treatment of Ciguatera.** Bull Soc Path Ex 1992;85:425-426.

Easom J, Fleming LE, Rowan A, Tamer, R, Wiersma S. **A Pilot Study of Harmful Algal Bloom Human Health Effects**. Proceedings of the International Harmful Algal Bloom 2000 Conference, Tasmania, **in press**.

Expert Panel. **Expert Scientific Review Panel on Pfeisteria: The Raleigh Report**, Water Resources Research Institute, State of North Carolina, RTP, NC, December 1997.

Fleming LE, Baden D, DeWailly E. **Ciguatera Epidemiologic and Public Health Case Study**. University of Miami NIEHS Marine and Fresh Water Biomedical Sciences Center, 2000.

Fleming LE, Baden D. **Harmful Algal Blooms and Human Health**. In: Oceans and Human Health. Knapp A et al., **in press**.

Fleming LE, Baden DG**. Neurotoxic Shellfish Poisoning: Public Health and Human Health Effects.** White Paper for the Proceedings of the Texas Conference on Neurotoxic Shellfish Poisoning, Proceedings of the Texas NSP Conference, Corpus Christi (Texas), April 1998:27-34.

Fleming LE, Bean JA, Baden D, Brams E. **Environmental health in the Caribbean**. Journal of Caribbean Studies 1997;12:6-22.

Fleming LE, Bean JA, Baden DG. **Epidemiology of Toxic Marine Phytoplankton**. In: UNESCO-IOC Manual on Harmful Marine Phytoplankton #33. Hallegraeff GM, Anderson DAN, Cembella AD. Paris: UNESCO, 1995, pgs. 475-488.

Fleming LE, Bean JA, Katz D, Hammond R. **The Epidemiology of Seafood Poisoning**. Hui, Kits, Stanfield. Seafood and Environmental Toxins. Marcel Dekker, 2000.

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Fleming LE, Blythe D, Baden D. **Marine Toxin Diseases: Ciguatera Poisoning.** Travel Medicine, 1997;1:1-4.

Fleming LE, Easom J, Baden D, Rowan A, Levin B. **Emerging Harmful Algal Blooms and Human Health: Pfeisteria and related organisms.** Toxicol Pathol 1999;27:573-581.

Fleming LE, Easom J, Steidinger K, Baden D. **VideoConference: Florida Harmful Algal Blooms (HABs): Human Health Effects**. Funded by CDC and Florida Dept of Health, Miami, FL, June 1999.(video, powerpoint presentation, hardcopy of powerpoint presentation with notes)

Fleming LE, Easom J. **Seafood Poisonings.** Travel Medicine 1998;2 (10):1-8.

Fleming LE, Stinn J. **Shellfish Poisonings.** Travel Medicine 1999;3:1-6.

Fleming LE, Washington G. **Scombroid Fish Poisonings.** Travel Medicine 1998;2(11): 1-5.

Fleming LE, Watkins S, Kaderman R, Levin B, Ayyar DR, Bizzio M, Stephens D, Bean JA. **Mercury Exposure in Humans through Food Consumption from the Everglades of Florida.** Water Air Soil Pollution 1995;80:41-48.

Fleming, L.E., Baden, D.G., Bean, J.A., Weisman, R., and Blythe, D.G. **Seafood toxin diseases: Issues in Epidemiology and community outreach**. In: Harmful Algae (B. Reguera, J. Blanco, M.L. Fernandez, and T. Wyatt, Eds.) Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO 1998, pp. 245-248.

Knap A, Dewailly E, Furgal C, Galvin J, Baden D, Bowen B, Depledge M, Duguy L, Fleming LE, Ford T, Moser F, Owen R, Suk W, Unluata U. **Indicators of Ocean Health and Human Health: A Research Framework.**Environmental Hlth Perspect, **in press**.

McKee D, Fleming LE, Tamer R, Weisman R. **Ciguatera Fish Poisoning Reporting by physicians in an endemic area**. Proceedings of the International Harmful Algal Bloom 2000 Conference, Tasmania, **in press**.

Stinn JF, De Sylva DP, Fleming LE, Hack E. **Geographic Information Systems (GIS) and ciguatera fish poisoning in the tropical Western Atlantic region**. Proceedings of the 1998 Geographic Information Systems in Public Health, Third National Conference San Diego, CA. http://www.atsdr.cdc.gov/gis/conference98/proceedings/html/stinn.html, 2000.

University of Miami NIEHS Center and NIEHS **1998 World Expo Touch Screen CD of Oceans and Human Health Issues**. Funded by the NIEHS, 1998.

Weisman R, Baden D, Fleming LE. **Ciguatera**. World Health Organization Emergency Response Manual. World Health Organization, Geneva, Switzerland, **in press**.

Pilot Project Program

Example #1

Pilot projects are an important component of the Center for Ecogenetics and Environmental Health. The goals of the pilot research project program are: 1) to stimulate CEEH investigators to initiate novel studies in the ecogenetics and related areas; 2) to attract University of Washington scientists not currently working in ecogenetics and related fields to this area; 3) to develop preliminary data to serve as a basis for future NIH research grants; and 4) to encourage collaborative and interdisciplinary work.

Pilot projects are advertised by circulars and various announcements to all CEEH investigators as well as to many University of Washington units with related activities.

Review of pilot projects consisted of enlisting one expert referee outside the University of Washington (UW) and one expert referee from inside the UW. An intense effort was made to obtain knowledgeable and critical reviewers by discussing the proposed project with informed local colleagues, if necessary. Personal telephone calls were found to be most effective in obtaining expert reviewers. The selected referees were asked to evaluate projects according to several criteria using NIH grading, ranging from 1-5. These criteria and their weighting were: scientific merit - 50%; relevance to the aims and goals of the CEEH - 25%; Collaborative and interdisciplinary involvement - 12.5%; and use of CEEH facilities - 12.5%. The reviews by the referees and the weighted ratings were further discussed by the CEEH executive committee in a meeting for final assessment and selection for funding.

CEEH received nine applications for the current funding year (2000-2001) and awarded four pilot grants at $20,000 each.

VII.A. Pilot Projects Funded in 2000 (04/01/00–03/31/01)

VII.A.1.
Title: Role of Glutamate-L-cysteine Ligase in Cystic Fibrosis

Investigator: Terrance J. Kavanagh, PhD, Associate Professor, Department of Environmental Health, UW

Description: Cystic fibrosis (CF) is a debilitating systemic disorder that affects the lungs, the pancreas and the intestine. Recently, it has been shown that CFTR may influence the ability of airway epithelial cells to transport the antioxidant glutathione. The invesigators have recently shown that a polymorphism in the GSH biosynthetic enzyme glutamate-L-cysteine ligase (GLCL) is associated with risk for idiopathic pulmonary fibrosis, a condition that also is characterized by low GSH in the alveolar lining fluid. This project will examine the prevalence of the GLCL polymorphism in CF patients, and assess the association of various alleles with disease severity. The investigators have obtained blood samples from over 70 CF patients from their collaborator Moira Aitken, Division of Pulmonary Medicine, and have devised a microsatellite genotyping method to amplify the GLCLC 5’ UTR from blood DNA obtained from these patients. They have determined that there is an statistically significant under-representation of the 9-GAG repeat allele in CF patients.

VII.A.2.
Title: Candidate Genetic Polymorphisms for Stroke in Young Women

Investigator: Stephen M. Schwartz, PhD, Associate Professor, Department of Epidemiology, UW

Description: The role of genetics in stroke is not well understood. This project is studying three candidate genes with known functional mutations suspected of being involved in the pathogenesis of ischemic and hemorrhagic strokes. Specifically, using data from a study of early-onset stroke in women from the Puget Sound metropolitan region, the proposed pilot study will 1) determine the prevalence of susceptibility alleles for the atrial natriuretic peptide gene (ANP) and two matrix metalloproteinase genes (MMP), and 2) obtain preliminary estimates of the association of polymorphisms in these genes with ischemic and hemorrhagic stoke. Subjects

were between the ages of 18 to 44 years with no previous history of clinical cerebrovascular or cardiovascular disease. A total of 41 ischemic stroke cases, 52 hemorrhagic stroke cases, and 250 controls have DNA available for genetic analysis. Assays have been developed and the CEEH Molecular Biomarkers Laboratory Facility Core is currently genotyping samples.

VII.A.3.
Title: Role of Platelet Thrombin Receptor (PAR1) Genetic Variants and Environmental Factors in the Susceptibility of Young Women to Acute Myocardical Infarction (MI)

Investigator: David S. Siscovick, MD, MPH, Professor, Departments of Epidemiology and Medicine, UW

Description: The investigators have previously demonstrated the importance of several coagulation factor and platelet receptor genetic susceptibility markers in combination with cigarette smoking in the risk of MI using a case-control study of MI in young women. This project will evaluate prothrombotic candidate genetic susceptibility markers and their interaction with environmental factors in the pathogenesis of early-onset athero-thrombotic disease. Thrombin is a multi-functional coagulation enzyme that is also important in platelet-dependent thrombosis following arterial wall injury. Several single nucleotide polymorphisms have been identified within the human platelet thrombin receptor gene (PAR1). The investigators have begun genotype association studies for selected PAR1 polymorphisms in a case-control study of acute MI in young women; they have also performed genotyping for a -506 promoter 13 bp insertion/deletion (I/D) polymorphism. The genetic influence on platelet thrombin receptor function will also be assessed through correlation of in vitro platelet thrombin aggregation response as well as platelet surface receptor levels with PAR1 genotypes.

VII.A.4.
Title: The Role of CYP209 Genotype in Modulating the Risk from Exposure to Exogenous Substrates: Warfarin and the Risk of Adverse Bleeding Events

Investigator: David Veenstra, PharmD, PhD, Assistant Professor, Department of Pharmacy, UW

Description: Warfarin is an oral blood-thinning agent used to prevent clotting events in patients with thromboembolic diseases. Its use is associated with significant risks of bleeding complications. Warfarin is metabolized primarily by CYP2C9, a major liver enzyme (cytochrome P450). Two variant alleles of CYP2C9, the *2 and *3 alleles, are associated with diminished CYP2C9 activity.

The investigators hypothesize that individuals with CYP2C9*2 or *3 alleles are more likely to have a higher risk of bleeding events due to impaired metabolism, experience greater variability in anticoagulation levels, and require lower maintenance doses of warfarin. They have begun a retrospective, case-control study to examine the association between CYP2C9 genotype and three clinical parameters: bleeding episodes, dose requirements, and anticoagulation variability. Results will be stratified based on smoking exposure. In January 2001, investigators began enrolling study participants, drawing from patients at UW Medical Center anticoagulation clinics. Full sequencing of exons 3 and 7 of the CYP2C9 locus, which contain the *2 and *3 mutation sites, has begun.

Example #2

PROJECTS FUNDED IN 2000

Year of Funding: 2000-2001
Title: Effect of Ureas and Like Agrochemicals on Gene Induction of Proteins Involved in the Immune Response. Investigators: D. A. Thompson, C. Morriseau, B. D. Hammock. Department of Entomology
Description: The immune response protects an organism both from a "hostile" environment replete with viral, fungal and bacterial challenges and from threats within the organism, eliminating cells deemed a threat to the organism. Due to human action, numerous chemicals, including agrochemicals, are now present in our environment. However, the effect of many compounds on the immune system is not well known. Recently, in a preliminary in vitro study, investigators observed that N-cyclohexyl-N'-dicyclohexylurea decreased the

production of interleukin 2 by Jurkat cells, an established cellular model of human T lymphocytes. This compound is a member of a new class of soluble epoxide hydrolase (sEH) inhibitors, which includes phenyl urea herbicides such as Diuron and Siduron and acylurea insecticides such as Dimilin (2). As interleukin 2 plays a critical role in the development of the T cell-based immune response, exposure to urea containing compounds may have downstream immune effects. This project hypothesizes that the observed inhibition of interleukin 2 production is linked to urea-induced sEH inhibition by an as yet unknown mechanism. Alternatively, the ureas may influence cytokine expression through altering patterns of gene expression in a sEH-independent fashion. The purpose of this study is to investigate these two complementary hypotheses.
Positive Outcomes: Too early for results.

Year of Funding: 2000-2001 and 1999-2000
Title: Probing Structure and Specificity Through Crystallographic Studies of Ligand Binding to the Aromatic Hydrocarbon Receptor
Investigators: Enoch Baldwin, Section of Molecular and Cellular Biology
Michael Denison, Department of Environmental Toxicology
Description: The overall goal of this proposal is to generate crystals of the ligand binding domain of the aromatic hydrocarbon receptor (AhR) for crystallographic analysis of the ligand binding domain of this important nuclear protein. The Ah receptor is the regulatory factor known to be responsible for mediating the toxic and biological effects of halogenated aromatic hydrocarbons and polycyclic aromatic hydrocarbons. The studies described in this proposal will take the first and most important step toward the characterization of the structure of the AhR ligand binding domain, the expression and crystallization of this AhR protein domain. These studies will not only allow future analysis of the mechanism of chemical-dependent activation of the AhR and facilitate predictions as to the types of chemicals which will interact with the AhR, but they will aid in the identification and/or designing of potential therapeutic agents for AhR-mediated toxic effects. Results are not listed here at the request of the investigators.
Positive Outcomes: Too early for results.

Year of Funding: 2000-2001 and 1999-2000
Title: Alteration of T Cell Signaling Produced by Immunotoxic Agents
Investigators: Margaret Yole, Department of Pharmacology/Toxicology; P. Richard Vulliet, Department of Molecular Biosciences
Description: The investigators propose that immunotoxicant binding of critical lymphocyte surface receptors or intracellular targets produces an aberrant non-polarized intracellular signal. It is believed this global signal prevents normal polarization of signaling machinery, cytoskeleton and secretory apparatus towards the area of contact formed between antigen-specific lymphocytes and complementary antigen presenting cells (APCs; B-cells, macrophages or dendritic cells). It is expected that, under most circumstances, in the absence of appropriate spatio-temporal stimuli by which to orient itself, the lymphocyte response to a toxicant-mediated stimulus will therefore be abortive. However, at certain optimum toxicant concentrations, the toxicant signal may mimic a polarized signal sufficiently to activate lymphocytes in non-antigen-specific fashion. It is anticipated that non-localized signaling may provide a basis for both the immunosuppressive and transient stimulatory and autoimmune effects of mercury.
Positive Outcomes: Too early for results.

Previous Pilot Projects 1997-98, 1998-99, and 1999-00

Year of Funding: 1999-2000
Title: CHIP-Based Expression of Analysis of Arsenate-Induced Carcinogenesis
Investigators: Jeffrey Gregg, M.D., Department of Pathology, UC Davis Medical Center
Robert Rice, Department of Environmental Toxicology
Description: The investigators will apply representational difference analysis (RDA) coupled to cDNA microarray technology to identify genes that are differentially expressed in human keratinocytes exposed to arsenate. The hypothesis is that arsenate affects certain fundamental regulatory pathways, leading to uncontrollable altered growth, and that these changes can be translated into differences in gene expression patterns with cDNA microarray assays. These expression patterns, or signatures, can be used to better understand the biology of arsenate-induced carcinogenesis and appropriate models then can be made to predict arsenate thresholds for carcinogenesis.

Positive Outcomes: Too early for results.

Years of Funding: 1998-1999, 1999-2000
Title: Pulmonary Tolerance In Vitro
Investigators: Laura Van Winkle, Department of Anatomy, Physiology and Cell Biology; Alan Buckpitt, Department of Molecular Biosciences; Charles Plopper, Department of Anatomy, Physiology and Cell Biology
Description: This pilot project will develop an in vitro model based on the in vivo model of naphthalene tolerance in mice. A newly defined organ culture system will be used. This system of bronchiolar explants has been previously demonstrated to undergo repair after injury and that retains metabolic activity in vitro. Explants of bronchioles are particularly relevant to agricultural exposures because distal bronchiolar epithelium is the primary target zone of P450-activated pulmonary toxicants, oxidant air pollutants and agricultural dusts. This project will use the bioactivated Clara cell toxicant naphthalene to establish this model because the in vivo production of tolerance by naphthalene in mice is well established (see Lakritz, et al 1996) and the Clara cell is the predominant cell type in the bronchiolar injury target zone. Naphthalene is a ubiquitous environmental pollutant (a byproduct of fossil fuels and their combustion, and a major component of sidestream tobacco smoke) that is also used in the synthesis of the pesticide carbaryl. The aim in these studies is to establish an in vitro model that reproduces the essential element of the tolerant phenotype; resistance to a challenge dose of naphthalene as determined directly by cytotoxicity assay and high resolution morphology. The long-term goal is to use this model to define the sequence of cellular and molecular events involved in the development of chemical tolerance. Results are not published here at the request of the investigators.
Positive Outcomes:
Peer Reviewed Publications:
1999 Van Winkle, LS, AR Buckpitt, ZA Johnson, SJ Nishio, CD Brown and CG Plopper. Early events in naphthalene-induced acute Clara cell toxicity: Comparison of membrane permeability and ultrastructure. Am. J. Resp. Cell Mol. Biol. 21: 44-53.
1999 Evans, M. J., L. S. Van Winkle, M. V. Fanucchi and C.G. Plopper. The attenuated fibroblast sheath of the respiratory tract epithelial-mesenchymal trophic unit. Am. J. Resp. Cell Mol. Biol. 21: 655-657.
2000 Evans, MJ, LS Van Winkle, MV Fanucchi, E Toskala, EC Luck, PL Sannes and CG Plopper. Three-dimensional organization of the lamina reticularis in the rat tracheal basement membrane zone. Am. J. Resp. Cell Mol. Biol. 22: 393-397.
Abstracts
1999 LS Van Winkle, CD Brown, ET Ocampo, JA Shimizu and CG Plopper. Bronchiolar epithelial cell morphology during repeated exposure to naphthalene in the mouse. Resp. and Crit. Care Med. 159 (3): A620
2000 LS Van Winkle, CD Brown, ET Ocampo, JA Shimizu and CG Plopper. Bronchiolar epithelial cell morphology during repeated exposure to naphthalene in vitro and in vivo. Resp. and Crit. Care Med 161:3 A172
Grants Awarded
Cystic Fibrosis Foundation (McDonald) “Airway EGFP Expression Following Aerosol Inhalation” $39,207/ yr 1 07/01/99-06/30/00
NIH RO1 ES04311 (Buckpitt) “Lung Injury by Naphthalenes” $203,425/ yr 1 07/01/99-06/30/04
CRPRC Base Grant Pilot Project #6 (Van Winkle) “Postnatal Airway Development and Allergen Exposure” 49,750/yr 1 05/01/00-04/30/02
Submitted Grants
TRDRP (Van Winkle) “Effect of Smoke and Gender on Bronchiolar Injury and Repair” $143,168/ yr 07/01/00-06/30/03

Year of Funding: 1999-2000
Title: Polychlorinated Biphenyls (PCBs) Promote Cell Cycle Progression in T-Cells by Altering Immunophilin (FKBP12, FK506 Binding Protein, 12 kDa)-Regulated Pathways
Investigators: Patty Wong-Yim, Department of Molecular Biosciences; Isaac Pessah, Department of Molecular Biosciences
Description: Several epidemiological studies indicate that developmental exposure to polychlorinated biphenyls (PCBs) represents a significant risk factor affecting parameters of learning and cognition in humans. The long-term objective is to understand exactly how the major ortho-substituted PCBs found in human tissues and their metabolites alter spatial and temporal aspects of Ca^{2+} signaling in neurons of hippocampus and how these mechanisms relate to alterations in (1) synaptic plasticity and (2) associative learning and memory. The

specific goals of this pilot proposal focus on understanding how PCB170 (2,2',3,3',4,4',5 heptachlorobiphenyl), affects neurodevelopmental toxicity in rats. Preliminary work has revealed that PCB170 is one of the most potent modulators of the ryanodine receptor (RyR2)gamma-immunophilin (FKBP12) Ca^{2+} channel complex which predominates within hippocampal neurons. Since the RyR2/FKBP12 complex regulates important aspects of neuroplasticity and has been shown to be closely associated with acquisition of spatial learning, the following hypotheses will be tested: 1) PCB170 is a potent modulator of the RyR2/FKBP12 complex of primary hippocampal neurons, an activity that alters short-term (functional) and long-term (transcriptional) Ca^{2+} signaling critical for neuroplasticity. 2) Perinatal exposure to PCB170 alters acquisition of hippocampal associative learning, an effect that is closely correlated with perturbations in the function and transcriptional induction of the RyR2/FKBP12 complex in CA1, CA3 and dentate gyrus. Methods and results are not published here at the request of the investigators.

Positive Outcomes: Too early for results.

Year of Funding: 1998-99

Title: Do Pesticides Modulate Linoleate Oxylipid Production and Toxicity in Kupffer Cells?

Investigators: Jiang Zheng, Department of Entomology; Richard Perez, UC Davis Medical Center Department of Surgery; Bruce Hammock, Department of Entomology; Charles Plopper, Department of Anatomy

Description: Despite the growing implication of leukotoxin in human pathophysiology, particularly in the pathogenesis of inflammatory diseases such as ARDS, it has not yet been determined whether leukotoxin or its diol metabolite are synthesized by inflammatory cells, whether cytokine production is modulated by these agents, nor whether epoxide hydrolase modulators, specifically phenyl or acyl urea-based pesticides and phenyl ether herbicides, have any effect on these activities. The overall goal of this project is to determine whether linoleate oxylipins are involved in the inflammatory response of Kupffer cells and whether the epoxide hydrolase inhibitors Dimilin and diuron, or the epoxide hydrolase stimulator, 2,4-D, modulate either Kupffer cell production of, or sensitivity to, cytokines and linoleate oxylipins. Effects of the above pesticides will be examined alone and in combination on the production, metabolism and toxicity of these oxylipins and correlate these data with cytokine levels as indicators of inflammatory response. This specific goal will complement the long-range goal of this laboratory in which is to understand how fatty acids are biosynthesized, metabolized and regulated in cells and will form the basis of collaborative grant proposals among the collaborating laboratories.

Positive Outcomes:

Publications

1. Draper AJ and Hammock BD. (1999) Soluble epoxide hydrolase in rat inflammatory cells is indistinguishable from soluble epoxide hydrolase in rat liver. Toxicol. Sci. 50, 30-35.
2. Newman JW, Denton DL, Morisseau C, Koger CS, Wheelock CE, Hinton DE, and Hammock BD (2000) Evaluation of fish models of soluble epoxide hydrolase inhibition. Environ. Health Persp. In press.
3. Nakagawa Y, Wheelock CE, Morisseau C, Goodrow MH, and Hammock BD. (2000) 3D QSAR analysis of inhibition of murine soluble epoxide hydrolase (MsEH) by benzoylureas, aryl ureas, and their analogs. Bioorg. Med. Chem. In press.

Grants

1. NIEHS RO1 ES02710-18 $325,450/5 years, Bruce Hammock, P. I.

Year of Funding: 1998-99

Title: Recombinant Peptides with Agrochemical Binding Specificities: Direct Selection from Phage Display Libraries

Investigators: Prabhakara Choudary, Department of Entomology; Alan Buckpitt, Department of Molecular Biosciences; Bruce Hammock, Department of Entomology

Description: The goal of this pilot project is to demonstrate that recombinant peptides with specific binding to agrochemicals can be isolated from phage-displayed random peptide libraries and that they can be effectively used in immunoassays and other antibody-based biological procedures. Success of this research will usher in a novel way of making antigen-binding molecules with a strong potential to replace conventional antibodies. Several benefits will accrue, including substantial savings in cost, time and human labor, increased stability and throughput of reagents and considerable reduction in animal sacrifice. The first year objectives are as follows:
I. Isolate recombinant phage displaying peptide(s) with binding specificity to simazine
II. Identify the consensus motif(s) of the peptides and the binding site residues critical for hapten binding

III. Validate hapten-binding activity of the peptides in immunoassay
Positive Outcomes: None as of yet.

Year of Funding: 1997-98

Title: Periimplantation biomarkers of human spontaneous abortion

Investigators: Bill Lasley, Department of Population Health; Daniel Jones, Facility for Advanced Instrumentation; Joseph Hill, Obstetrics and Gynecology, Harvard University School of Medicine

Description: This is a collaborative project on the endocrinology of early human pregnancy and spontaneous abortion (SAB). Dr. Lasley has funding from NIEHS to develop and apply assays for urinary hormone metabolites that can be used in prospective epidemiologic field studies of reproductive hazards in the environment. In the course of this research, investigators in the NIEHS Center have developed assays for metabolites of estradiol, progesterone, LH, FSH and hCG. They carried out clinical studies with infertility patients to validate the assays before taking them to the field. Many of the clinical studies involve collection of daily blood and urine samples which enable the investigators to compare the pattern of secretion of the circulating hormone with the pattern of metabolite excretion. The investigators have done a number of such studies on pregnant women and have daily samples from a number of conception cycles which ended in SAB as well as many normal pregnancies. In current studies, several immunoassays are used to detect intact hCG as well as other hCG beta-related molecules. Investigators also have a radio-receptor assay (RRA) and a bioassay, both of which utilize fetal kidney cells (or cell membranes) following transfection with the message for the human LH/hCG receptor.
Positive Outcomes:
Program Project Renewal NIH 5-P01-ES-06198, Interdisciplinary Studies in Reproductive Toxicology and Epidemiology $3,290,755;
Publications:
1. Guo Y, Hendrickx AG, Dieter J, Lasley BL, Stewart DR, Tarantal AF, Overstreet JW. Biomarkers of early fetal loss in macaques: TCDD as a model of environmental toxicity, Biology of Reproduction 60(3):707-713, 1999. 2. Lasley, B.L., and Overstreet, J.W. (1998). Biomarkers for human reproductive health, an interdisciplinary approach. Env Health Perspectives 106:955-960.

Year of Funding: 1997-98 and 1998-99

Title: Developing a Mouse Model for Studies on Heritable Effects of Male Methamidophos Exposure

Investigators: Lynn Wiley, Obstetrics and Gynecology; James Overstreet, Obstetrics and Gynecology; Barry Wilson, Animal Science and Environmental Toxicology; Dena Towner, Obstetrics and Gynecology; Terrance Hassold, Department of Human Genetics and the Center for Human Genetics, Case Western Reserve University, Cleveland, Ohio

Description: This pilot project addresses the question of whether methamidophos exposure to the paternal germline can lead to transmitted effects in the progeny and whether these effects are heritable across generations. As a class of compounds, exposure to organophosphate pesticides results in chromosomal aberrations in lymphoid- and bone marrow cells in humans, rats and mice. Methamidophos in particular induces chromosomal aberrations in cultured mouse bone marrow cells. These observations indicate that organophosphates including methamidophos are capable of producing cytogenetic damage in mammalian cells. At this time there is no published information from animal models on whether methamidophos can also cause cytogenetic damage in mammalian gametes (sperm cells). The organophosphate fungicide/ insecticide Hinosan (O-ethyl, S,S-diphenyl phosphorodithioate), which is used extensively to treat post-harvest rice fields, causes abnormalities in sperm parameters of exposed mice as does methyl parathion. However, it is not known whether paternal exposures to these pesticides cause cytogenetic damage in sperm and/or transmitted/heritable effects. The overall objective is to determine whether biomarkers that can be measured in fathers' semen are predictive of a risk for transmitted and heritable health effects that are not easily measured in children and/or may be manifested later in their adult life.
Positive Outcomes: R01 Renewal ES-06516, Heritability of Embryonic Radiosensitive Targets, $1.4M;
Publication: Burruel VR, Raabe OG, Overstreet JW, Wilson BW and LMWiley. 2000. Paternal effects from methamidophos administration in mice. Toxicology and Applied Pharmacology 165:148-157.